THE NIJMEGEN BREAKAGE SYNDROME

clinical, immunological, cytogenetic and cellbiological studies

ROB TAALMAN

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PROEFSCHRIFT

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Ter herinnering aan mijn vader
aan mijn moeder
aan Theone

Contents

Chapter 1

INTRODUCTION

More than thirty years ago Tjio and Levan (1956) and Ford and Hamerton (1956) demonstrated that human somatic cells have 46 chromosomes, 22 pairs and the sex chromosomes, either XX or XY. Following this discovery, chromosome identification techniques were quickly applied to clinical practice and during the next half dozen years the causes of clinical syndromes such as Down's syndrome, Turner syndrome and Klinefelter syndrome were established (Lejeune, 1959; Ford et al, 1959; Jacobs and Strong, 1959). The development of banding techniques in the ensuing years have contributed considerably in further progress of human genetics. By the use of various dyes and treatments, mitotic chromosomes can be visualized as having characteristic banding patterns facilitating identification of individual chromosomes (Caspersson, 1971; Seabright, 1971; Dutrillaux and Lejeune, 1971; Scheres, 1976). This improved resolution has resulted in the delineation of more subtle chromosome disorders (Schinzel, 1984). The phenomenon of spontaneous chromosome instability was first reported in the mid 1960's. Spontaneously increased chromosome breakage is well documented now in three autosomal recessive inherited diseases: Bloom's syndrome (BS)(German et al, 1965), Fanconi's anemia (FA)(Schroeder et al, 1964), and ataxia telangiectasia (AT)(Hecht et al, 1966). The chromosome constitution of such patients is basically normal (46,XX or 46,XY). However, chromatid breaks, chromosome breaks, fragments, translocations and more complex rearrangements may be present in a considerable number of their cultured cells. Because of these features the disorders were designated as chromosome breakage

syndromes. Since the term 'breakage' is not uniformely applicable to all these syndromes, they are also designated as the chromosome instability syndromes. Because the Nijmegen breakage syndrome that is to be presented in this thesis shows affinities with the socalled classic instability disorders Bloom's syndrome, Fanconi's anemia and ataxia telangiectasia it is desirable to describe these disorders shortly.

Bloom's syndrome

Bloom's syndrome (Bloom, 1954; German et al, 1965; German et al, 1984) is characterized by microcephaly, growth retardation. The face is small and narrow. The skin of these patients is sun sensitive and erythema may appear over the malar areas, especially after exposure to the sun. The mental development is usually normal. Adult males appear to be infertile and women conceive only rarely. Many patients have serious respiratory and intestinal infections owing to impairment of the immune system. Both the humoral and cellular immunity is disturbed (Weemaes et al, 1979). A drastically increased risk of developing cancer at early age exists (German et al, 1979; German et al, 1984). BS exhibits an elevated incidence of chromosome aberrations and an increased frequency of sister chromatid exchanges. Chromosome instability has been observed in cultured lymphocytes, bone marrow and dermal fibroblasts. The most characteristic chromosomal aberration is the symmetrical quadriradial, which is the result of mitotic crossing over between homologous chromosomes (German et al, 1974; Schroeder and German, 1974;

Therman and Kuhn, 1981; Kuhn and Therman, 1986). Another cytogenetic feature which is very typical for BS is the striking increase (10- to 14-fold) of sister chromatid exchange (SCE) frequency (Chaganti et al, 1974; Kuhn and Therman 1986). An elevated frequency of SCE's is now considered diagnostic for Bloom's syndrome (Hustinx et al, 1977).

Fanconi's anemia

Fanconi's anemia is associated with progressive marrow failure of all marrow elements (Reinhold et al, 1952; Schroeder, 1964; Fanconi, 1967). Congenital abnormalities such as skeletal anomalies and renal defects are also characteristic. Other common features are skin hyperpigmentation, small stature and mild mental retardation. There is a considerable variation among FA patients in both the congenital abnormalities and the age of onset of the pancytopenia. While the majority of FA deaths results from hemorrhage or infections, a significant portion of the patients develop malignancies (German, 1979,1980; Schroeder, 1982). The increased level of spontaneous chromosomal breakage and rearrangements in cultured cells is a consistent finding in essentially all FA patients and is often used to confirm the diagnosis of FA. There is inter- and intraindividual variability in the frequency of aberrant cells (Schroeder et al, 1976; McIntosh et al, 1979). The cytogenetic disturbance in FA includes chromatid- and chromosome gaps, chromatid- and chromosome breaks, triradial and quadriradial interchanges and dicentrics. The quadriradials in cells from FA patients are, in contrast to BS,

mostly asymmetrical (interchanges between nonhomologous chromosomes). Cells from FA patients are particularly susceptible to chromosomal aberration induction by polyfunctional alkylating agents (Schuler et al, 1969; Sasaki and Tonomura, 1973; Latt et al, 1975). Auerbach et al (1981) suggested that induction of chromosomal damage by crosslinking agents is closely related to the basic defect in FA and that the diagnosis FA can be made only in patients whose cells show the genotoxic action of crosslinking agents. Only the elucidation of the molecular defect in FA can verify if this definition is correct.

Ataxia telangiectasia

The predominant clinical features of AT are progressive cerebellar ataxia, telangiectasias especially in the bulbar conjunctiva and increased sinopulmonary infections (Louis-Bar, 1941; Boder and Sedgwick, 1957; for a review see Boder, 1985). Hypogonadism may also be prominent and in about 75% of the patients growth retardation occurs. Ataxia is always the first and presenting symptom, and usually becomes apparent as the child begins to walk. In general, telangiectasia has later onset than the ataxia. Patients with this disorder are unusually susceptible to infections, particularly sinopulmonary infections often cause death in childhood and adolescence. Immunodeficiency affects both cellular and humoral immune systems. There is an almost consistent elevation of the serum alpha-fetoprotein, suggesting an abnormal maturation of the liver (Waldmann and McIntire, 1972).

About 15% of the AT patients develop malignancies, mainly affecting lymphoreticular tissue (Spector et al, 1978).
The existence of chromosome instability in AT was first detected by Hecht and coworkers (1966). Chromosome aberrations found in AT cells include chromatid- and isochromatid gaps and breaks, acentric fragments, structurally rearranged chromosomes in a high frequency and also in a low frequency triradial and quadriradial interchange figures. The most characteristic cytogenetic feature is the frequent occurence of (clones with) typical chromosome 14 (Cohen et al, 1973) and 7 (Aurias et al, 1980; Scheres et al, 1980) translocations.
Patients with AT show a clinical hypersensitivity to X-rays. Several cases of AT patients are reported who developed severe acute radiation damage after treatment for a malignancy with conventional doses of radiation (Gotoff et al, 1967; Miller, 1982; Pritchard et al, 1982).
This clinical observations have lead to the exploration of the in vitro hypersensitivity to X-rays in AT. It was shown that in cultured AT cells the radiosensitivity is expressed by diminished cell survival (Taylor et al, 1975), by enhanced chromosome breakage (Taylor et al, 1976) and by a reduced inhibition of the DNA-synthesis (de Wit et al, 1981).

OUTLINE OF THE THESIS

The coincidence of chromosome instability and immunodeficiency in the afore-mentioned cancer prone diseases provides a unique

occasion to study the relationships between chromosomal damage, carcinogenesis and immunological impairment. For this reason the chromosome instability disorders have been one of the main interests of the departments of Human Genetics and Pediatrics from the University of Nijmegen for a long time (Hustinx et al, 1977; Weemaes et al, 1979; Scheres et al, 1980; Jaspers et al, 1981). Any patient suspected for having a chromosome instability syndrome was extensively investigated on spontaneous chromosome instability, sister chromatid exchanges, hypersensitivity to clastogenic agents and immunological defects, uncovering twenty-nine probands with a chromosome instability disorder. In one of these patients, a boy, who was investigated because of his immunodeficiency, a conspicuous karyotype instability was found (Hustinx et al, 1979). However, his clinical disorder did not fit to any of the classic chromosome breakage syndromes. To describe this 'new' disorder, further clinical, immunological and cytogenetic studies were performed in this proband and his family. His main clinical characteristics were: microcephaly, stunted growth, mental retardation, cafe au lait spots and recurrent respiratory infections. An older brother of the proband has had basically the same clinical symptoms but his immunological disturbance seemed to have been more severe. In both sibs there was an IgA-deficiency and the antibody responses were disturbed. Cytogenetic studies were carried out on bone marrow, cultured lymphocytes and fibroblasts of the proband and typical chromosome 7 and 14 translocations appeared to be present in cultured lymphocytes only. Also in the relatives such chromosomal abnormalities, though in low frequencies, were found. The similarity of the clinical symptoms in both sibs, the

cytogenetic findings in the family and the consanguinity of the parents suggest that the disorder has a recessive mode of inheritance. Although the immunological and cytogenetic data in this disorder resemble those in ataxia telangiectasia, the diagnosis AT could not be made because the cardinal symptoms cerebellar ataxia and oculocutaneous telangiectasias were lacking. There were also no indications that the patient was suffering from one of the other chromosome breakage syndromes, BS or FA. Therefore, the disorder was considered to be a new chromosomal breakage syndrome which was given the provisional name the Nijmegen breakage syndrome (NBS)(Chapter 2).

Because NBS showed some resemblance with AT, the radiosensitivity of the probands cells was investigated and compared with the hypersensitivity of AT-cells to X-rays. The cells from the NBS patient appeared to be hypersensitive to ionizing radiation and also to the radiomimetic drug bleomycine. After treatment with X-rays or bleomycine the cell survival was diminished, while the DNA synthesis in NBS cells turned out to be less inhibited than in normal cells. In both cultured lymphocytes and fibroblasts chromosome damage was increased following ionizing radiation. The reaction of NBS cells which was similar to that of cells of ataxia telangiectasia patients, indicates that both syndromes must be closely related (Chapter 3).

On account of the remarkable similar response of NBS and AT cells to X-rays, cytogenetic and immunological studies were performed in a number of AT patients. A wide variability in the level and type of chromosome aberrations was found in cultured cells of the

AT patients. The immunoglobulin levels and the percentage of T-cells varied from patient to patient. Because of this diversity, both the immunological screening and the cytogenetic analysis cannot be of help in exclusion of the diagnosis AT. For early diagnosis, when both cerebellar ataxia and telangiectasia is not yet manifest, the reduced inhibition of the DNA-synthesis after exposure to ionizing radiation might be of diagnostic importance (Chapter 4).

Since the first description of the probands with NBS (Chapter 1) we have been able to study four other families with apparently the same syndrome. Three of the families were reported earlier as having a possible new syndrome with microcephaly, immunodeficiency and risk for lymphoreticular malignancies (Seemanová et al, 1985). In these patients from Czechoslovakia and also in another patient from the Netherlands all NBS features, as described earlier, were present. Clinical, cytogenetic, cellbiological and immnological data, from these patients, were evaluated for a further delineation of the syndrome. (Chapters 5 and 6).

In view of the cellular analogies between NBS and AT a genetic study of the syndromes has been performed through complementation analysis. This analysis was carried out in fused cells using the radioresistant DNA-synthesis as a marker. None of the NBS cell lines tested belonged to one of the presently known complementation groups of AT. In addition NBS appeared to be genetically heterogeneous itself since two NBS-complementationgroups were established. It is concluded that

NBS patients suffer from a chromosome instability disorder that is distinct from AT (Chapter 7).

The most common immunoglobulin deficiency in NBS and AT is IgA deficiency. Three of our NBS patients have an IgA deficiency while the fifth has a decreased level IgA (Chapters 2 and 6). From the AT patients reported in Chapter 4, four out of six had a marked IgA deficiency. Studies performed by Rivat-Peran et al (1981) suggested that 60% of the AT patients have decreased IgA levels and that in the remaining 40% normal IgA1 concentrations mask the IgA2 deficiencies in allmost all AT patients. The aim of the experiments described in Chapter 8 was to study the relation of IgA-deficiency, chromosome instability and hypersensitivity to X-rays. For this purpose cytogenetic studies were performed in patients with a selective IgA-deficiency.

It is well known that specific sites in the human genome are prone to chromosomal breakage and rearrangements (Yunis and Soreng, 1984; LeBeau 1986). In both NBS and AT four chromosomal sites are preferentially involved in the chromosome 7 and 14 rearrangements. The same translocations are also seen, though in a much lower frequency, in cultured lymphocytes of normal persons (Scheres et al, 1986; Hecht et al, 1987). It has been proposed that breakage at those specific sites, leading to the typical translocations, might be a consequence of intrinsic rearrangements in relation to immunological maturation (Scheres et al, 1986; Hecht et al, 1987). Because sister chromatid exchanges are thought to reflect cellular recombinational activity we studied if these sites are also more involved in

processes of sister chromatid exchange (Chapter 9).

In the last chapter an attempt will be made to postulate a unifying concept for the radiation sensitive disorders AT and NBS (Chapter 10).

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Chapter 2

A NEW CHROMOSOMAL INSTABILITY DISORDER: THE NIJMEGEN BREAKAGE SYNDROME

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A NEW CHROMOSOMAL INSTABILITY DISORDER: THE NIJMEGEN BREAKAGE SYNDROME

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ABSTRACT. Weemaes, C. M. R., Hustinx, T. W. J., Scheres, J. M. J. C., van Munster, P. J. J., Bakkeren, J. A. J. M. and Taalman, R. D. F. M. (Departments of Paediatrics and Human Genetics, University of Nijmegen, Nijmegen, The Netherlands.) Acta Paediatr Scand, 70: 557, 1981.—A 10-year-old boy with microcephaly, stunted growth, mental retardation, café-au-lait spots and immunodeficiency is described. An older brother of the patient had the same clinical symptoms and a more severe immunodeficiency. Cytogenetic studies in the proband revealed a typical form of chromosome instability with multiple rearrangements of chromosomes 7 and 14. Such abnormalities were also present, though in very low frequencies, in the father and three of the phenotypically normal sibs. The similarity of the symptoms in the two sibs, the close consanguinity of their parents and the results of the cytogenetic studies in the family favour the hypothesis that the disorder is an inherited one. The clinical features and the chromosome aberrations as present in the proband are usually found in chromosomal breakage syndromes, but it was possible to exclude each of the classical chromosomal breakage syndromes on clinical and/or cytogenetic grounds.

KEY WORDS: Chromosomal breakage syndrome, immunodeficiency, mental retardation

An abnormal tendency of the chromosomes to break and rearrange is found in cultured cells from patients with Bloom's syndrome, ataxia telangiectasia, Fanconi's anaemia and, after UV-irradiation also in cells from patients with xeroderma pigmentosum (1, 2). Clinically and cytogenetically these autosomal recessive so-called breakage disorders can clearly be identified as separate entities, but they share a number of features, viz. stunted growth, skin abnormalities, a tendency towards malignancy and immunological disturbances (1, 2, 3, 4). Recently we described typical chromosomal abnormalities in a patient with such symptoms (5). Further clinical, immunological and cytogenetic investigations have since been performed in the patient and in the family. The results strongly suggest that this disorder represents a new chromosomal breakage syndrome.

METHODS

Routine chromosome anlyses were performed on peripheral blood lymphocytes stimulated with phytohaemagglutinin (PHA). The cells were cultured in R.P.M.I. 1640 medium supplemented with 20% fetal calf serum. The proband's lymphocytes were also cultured after stimulation with pokeweed mitogen (PWM). In addition, his bone marrow cells and cultured skin fibroblasts were cytogenetically analysed. Staining of chromosome preparations was performed using Scheres' (6) modification of Seabright's (7) G-banding technique. The frequency of spontaneous sister chromatid exchanges was determined with the method of Scheres et al. (8).

Immunoglobulin concentrations were determined by an immunoturbidimetric method using an LKB reaction rate analyser (9). Tetanus titrations were performed according to Ipsen (10) as were diphtheria titrations (11), and polio titrations with the methods of Salk et al. (12). Antibodies to Helix pomatia haemocyanin (HPH) were kindly determined by Dr H. The (State University Groningen, Dept. Int. Med., Div. Immunol., Groningen) (13). Lymphocyte stimulation was measured according to DuBois et al. (14). The cells were incubated with PHA (PHA-P, Difco), with PWM (Gibco) or with one of the following antigens: tetanus toxoid, diphtheria toxoid (both a gift from Dr J. Nagel, R. I. V. Bilthoven), *Candida albicans* (Hollister Stier Laboratories) or HPH (a gift from Dr H. The, Groningen). Mixed lymphocyte reactions (MLR's) were carried out as described previously (15).

E-Rosette tests were done after Stjernsward et al. (16). Immunofluorescence studies were performed according to the method of Vossen (17) with a slight modification (15).

CASE REPORTS

Case 1. The proband H. H., a boy, was born in 1969. Pregnancy duration was 38 weeks and birth weight 2500 g

Fig. 1. The proband H. H. at the age of 9.

Fig 2. The proband's brother (case 2) at the age of 6.

(=P 10). When he was 6 weeks old his head circumference measured 33 cm (<P 3).

During infancy he suffered from repeated upper respiratory tract infections and he had one attack of bronchitis. Tonsillectomy and adenoidectomy were performed at the age of 6. Chickenpox and measles were handled normally. On physical examination at the age of 9 (Fig. 1) a microcephalic small boy was seen (length 124 cm (just below P 3), weight 21 kg (<P 3), head circumference 45 cm (<P 3). He had a weak sun-sensitive erythema in the face. When exposed to the sun in summer he complained of painful eyelids. He had many freckles and on the trunk there were seven café-au-lait spots and two vitiligo patches. No telangiectasias were seen. He was mentally retarded (IQ 67). Neurological examinations did not reveal further abnormalities. The hemoglobin was 7.8 mmol/l and the white blood cell count was $5\,300\times10^6$/l. The platelet count was 216×10^9/l. Renal functions were normal. IVU revealed no abnormalities.

Case 2. An older brother, M. H. (Fig. 2) was born in 1964 at term. Birth weight was 2 500 g (<P 3). His length and weight, though gradually increasing, remained subnormal (<P 3). He was microcephalic (<P 3) and mentally retarded. He had a large café-au-lait spot on the trunk (cross section 4 cm). From his first year onwards he had suffered from otitis media and repeated pneumonias. He had also had an oral candida infection. At the age of $1\,^1/_2$ a urinary tract infection occurred and the left kidney showed hydronephrosis. When he was 6 years old a nephrectomy was performed. At the age of $4\,^1/_2$ a hypogammaglobulinemia was found and gammaglobulin therapy was started. Despite of this he sustained draining ears and repeated episodes of diarrhoea. At the age of $5\,^1/_2$ the course of a chickenpox infection was very severe but he recovered. When he was $6\,^1/_2$ he was hospitalized for an interstitial pneumonia but did not respond to antibiotic and plasma therapy and he died from respiratory failure.

At autopsy massive infiltration of the lungs was found. The thymus appeared morphologically normal. In the gut Peyer's patches were absent. The cerebrum was too small with signs of atrophy. The cerebellum was normal.

Family data. The parents who are second cousins, and the four sibs still alive are phenotypically normal (Fig. 3). The third child was born after a pregnancy duration of 7 months (birth weight 1 850 g) and died after 4 hours. No clinical data are available. There does not appear to be any familial predisposition towards malignancy.

CYTOGENETIC STUDIES

Some preliminary results of a chromosome study in the proband have already been described (5), but since then important new cytogenetic observations have been made in the patient and the family members. In PHA-stimulated lymphocytes of the proband a remark-

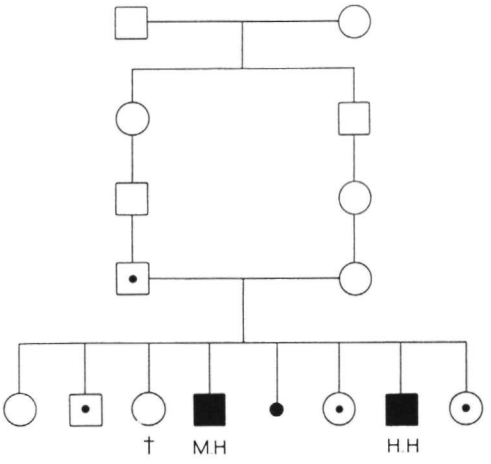

Fig. 3. Parental consanguinity and sibship of the proband H.H. □ = male, ○ = female, • = abortion; marker chromosomes were present in the family-members labeled with a dot.

able mosaicism was detected: 15.9% of the metaphases showed structural chromosome aberrations which involved one, two or even three of the chromosomes Nos. 7 and 14. Most of the rearrangements were reciprocal translocations and the exchange points were exclusively located in four "fragile" sites: two in chromosome No. 7 (7p13 and 7q32) and two in chromosome No. 14 (14q11 and 14q32) (Fig. 4).

The most frequently observed aberrations were a pericentric inversion of a chromosome No. 7, two different reciprocal translocations between a chromosome No. 7 and a chromosome No. 14, and a reciprocal translocation between both chromosomes No. 7 (Fig. 5). There were also a number of less frequent types of 7/7 and 7/14 translocations, including some complex forms in which both chromosomes No. 7 and a chromosome No. 14 participated. In one metaphase a 14q/14q tandem translocation was seen which was identical to the Dq+ marker chromosome reported to be characteristic for ataxia telangiectasia (18). Until now ten different 7/7, 7/14 and 14/14 translocations have been seen in PHA-stimulated lymphocytes of our patient, and all involved two or more of the "fragile sites" mentioned above. In addition, a few translocations between a chromosome number 7 or 14 and one other autosome were present, but they were very rare. Nevertheless they invariably involved one of the four "hot spots".

Unspecific chromatid breaks occurred in about 6% of the cells. A triradial configuration was seen once, as was an isochromosome 9q. In PHA-stimulated lymphocytes the number of sister chromatid exchanges (SCE's) per cell was 12.4±4.1 (S.D.) which is not increased as compared with the value in our controls (12.0±3.3).

In PWM-stimulated cells the typical 7 and 14 marker translocations were also seen, but not in direct bone marrow preparations nor in cultured skin fibroblasts (Table 1). In one fibroblast metaphase, however, a chromatid break had occurred at the fragile 7q32 site.

The chromosomes of the parents and the living sibs were also studied. The mother had a normal chromosome pattern in 115 cells, and the father had a typical 7/14 translocation in one out of 300 cells. The oldest brother of the proband and the two youngest sisters each had one cell out of 100 with a translocation identical to one of the markers in the proband viz. a 14q/14q, a 7/14 and a 7/7 translocation, respectively (Fig. 6).

IMMUNOLOGICAL STUDIES

Quantitative serum immunoglobulin studies revealed normal serum IgM levels in both pa-

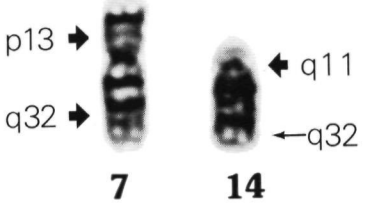

Fig. 4. The four "fragile sites" in the chromosomes Nos. 7 and 14 of the proband.

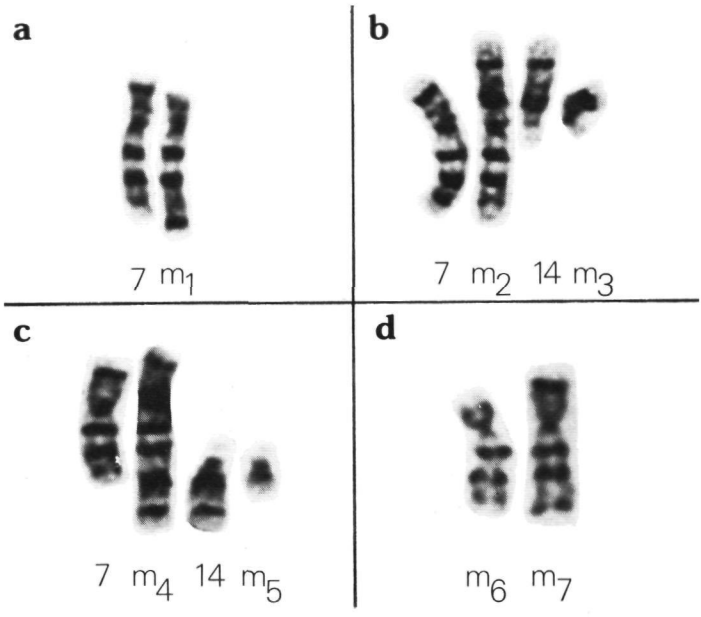

Fig. 5. The four most frequent chromosome Nos. 7 and 14 rearrangements in the proband's lymphocytes. (*a*) Pericentric inversion of chromosome 7, inv(7)(p13q32), resulting in marker chromosome m_1. (*b*) Reciprocal 7/14 translocation t(7;14)(p13;q11) resulting in marker chromosomes m_2 and m_3. (*c*) Reciprocal 7/14 translocation t(7;14)(q32;q11) resulting in m_4 and m_5. (*d*) Reciprocal 7/7 translocation t(7;7)(p13;q32) resulting in m_6 and m_7.

tients (Table 2). The IgG level was normal in case 1 but greatly decreased in case 2. Both patients had an IgA deficiency. In case 1 IgE was also deficient (case 2 was not tested). In the parents and the four sibs the serum immunoglobulin levels were normal (Table 2). Serum isohemagglutinin titres were normal in both patients (anti-B 1/256 in H. H. and 1/32 in M. H.). Both patients were challenged with a booster of diphtheria and tetanus toxoids and poliomyelitis vaccin. After 14 days the proband's response was normal but his brother showed no reaction to diphtheria and tetanus toxoids and only a subnormal rise in the level of antibodies to poliomyelitis vaccin (titre 1/4). No antibody response was seen in the proband after injection with HPH (Table 3). His in vitro lymphocyte responses to phytohaemagglutinin, pokeweed mitogen and to allogeneic lymphocytes were normal. The lymphocytes showed low responses to common microbial antigens (tetanus toxoid, diphtheria toxoid and *Candida albicans*) and no response to HPH 2 weeks after immunization. The percentage of cells bearing surface immunoglobulins (B lymphocytes) and of E-rosette forming cells (T lymphocytes) were within the normal range.

DISCUSSION

The two brothers show a similar clinical picture: microcephaly, stunted growth, mental

Table 1. *Frequencies of the most typical rearrangements in several cell types of H. H.*
(number per 100 cells)

Cell type	inv(7) (p−;q+)	t(7;14) (p+;q−)	t(7;14) (q+;q−)	t(7;7) (p−;q+)	All 7 and 14 rearrangements
Lymphocytes (PHA), n=334	6.9	3.6	2.7	1.2	15.9
Lymphocytes (PWM), n=129	4.7	4.7	0.8	1.6	12.4
Bone marrow, n=50	–	–	–	–	–
Fibroblasts, n=80	–	–	–	–	–[a]

[a] One cell with a chromatid break at 7q32 was seen.

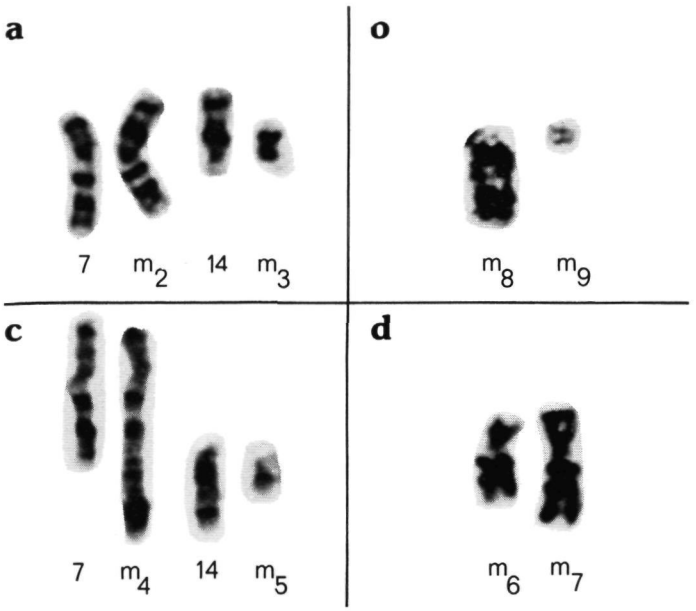

Fig. 6. Chromosome 7 and 14 rearrangements found in the proband's father (*a*), the oldest brother (*b*), an older sister (*c*) and the youngest sister (*d*). Marker chromosomes m_2–m_7 are identical to those given in Fig. 5; m_8 and m_9 resulted from a reciprocal tandem 14/14 translocation t(14;14)(q11;q32) which was also found in the proband but not shown in Fig. 5.

retardation and café-au-lait spots. They have a serious immunodeficiency. Both have a pronounced IgA deficiency. In addition, the proband has a disturbed primary immune response. His older brother had a more severe defect in humoral immunity: the serum IgG level was decreased and the antibody response after booster immunization was found to be greatly diminished.

Cytogenetic studies in the proband revealed many lymphocyte metaphases with typical chromosome 7 and 14 translocations. As a marked humoral immunodeficiency exists in our patients we investigated whether the chromosomal instability was mainly localized in the B-lymphocytes, which are the precursors of the antibody producing plasmacells. However, the experiments with PHA and PWM seem to indicate that the marker translocations are present in both T and B cells.

The remarkable similarity of the clinical symptoms, the parents' close consanguinity and the cytogenetic findings in the family suggest that the disorder in our patients is an inherited and probably recessive one. The clinical features, the immunodeficiency and chromosome aberrations such as seen in the proband are typical of patients with a chromosomal breakage syndrome (Table 4) (1, 2). However, the disease in our patients can clearly be discerned from each of the classical breakage syndromes. Clinical evaluation excluded xeroderma pigmentosum (no skin or eye aberrations) and Fanconi's anaemia (no pancytopenia, no skeletal deformities). Bloom's syndrome, which might have been suggested by the microcephaly, the stunted

Table 2. *Serum immunoglobulin levels in the patients, the parents and the sibs (IU/ml)*

	IgM	IgG	IgA	IgE	IgD
HH					
9 years	71	127	<1	<5	
11 years	66	109	<1	<5	<10
MH					
5 years	150	34	<1		
Father	127	123	172	33	89
Mother	84	110	81	11	42
Brother	103	129	110	112	62
Oldest sister	132	128	138	175	132
Second sister	131	122	51	12	
Youngest sister	72	107	46	83	34

Table 3. *Class specific antibody titres (Elisa) against the primary immunogen Helix pomatia haemocyanin (HPH) in case 1 (H. H.)*

	Anti-HPH titre		
	IgG	IgM	IgA
Before immunisation	<20	40	<20
2 weeks after immunisation	<20	80	<20
6 weeks after immunisation	<20	20	<20
Positive control	1 280	2 560	2 560
Negative control	<20	160	<20

growth and the facial erythema of the proband can also be excluded because there are no typical quadriradial chromosome rearrangements and the SCE frequency is not increased (19, 20).

The diagnosis ataxia telangiectasia was strongly suggested by the results of the cytogenetic studies in the proband: multiple chromosome 14 aberrations (including the tandem 14q/14q translocation) are typical of this disease (18). In ataxia telangiectasia these rearrangements are, however, also present in cultured skin fibroblasts, which seems not the case in our patient. Furthermore, the same or presumably the same typical chromosome 7 inversions and 7/14 translocations as in our patient have recently been recognized as being non-random aberrations in patients with classical ataxia telangiectasia (22, 23): the break points indicated by Aurias et al. (23) differ slightly from those found by us (22), but this is probably the result of the different staining techniques used. Also, preliminary studies have revealed an increased sensitivity of our proband's lymphocytes to X-rays, but which is less than that found in patients with ataxia telangiectasia (Scheres et al., to be published). In addition, the immunological data resemble those in ataxia telangiectasia as especially IgA-, but also IgE-deficiency, decreased serum IgG levels and disturbances in antibody responses have been described in this syndrome (24, 25). Nevertheless, the diagnosis ataxia telangiectasia cannot be made in our patients because they have neither ataxia nor oculocutaneous telangiectasia. In all variants of this syndrome, hitherto known, ataxia is

Table 4. *Comparison of the symptoms of the patients with those of the classical breakage syndromes*

	Bloom's syndrome	Ataxia telangi-ectasia	Fanconi's anemia	Xeroderma pigmento-sum	Our patients	
					H. H	M. H.
Intrauterine growth retardation	+	−	+		±	±
Stunted growth	+	+	+	+	+	+
Microcephaly	+	−	+		+	+
Mental retardation		+	+	+	+	+
Telangiectasia						
Conjunctival	−	+			−	−
Elsewhere	+	+		+	−	−
Skin abnormalities						
Sun sensitivity	+	−		+	+	
Freckles				+	+	
Café-au-lait spots	+	+	+		+	+
Vitiligo spots	+	+			+	
Progeric changes		+		+	−	
Cerebellar abnormalities	−	+	−	−	−	−
Pancytopenia	−	−	+	−	−	−
Renal anomalies	−	−	+		−	+
Skeletal deformities	+		+		−	−
Eye disorders	−			+	−	−
Defect in immunity	+	+	+	+	+	+
Infections	+	+			−	+
Tendency toward malignancy	+	+	+	+		

already present in early childhood (2 26) and oculocutaneous telangiectasias are usually first observed before the age of 7 years (26)

We therefore strongly suggest that we have detected a new chromosome breakage disorder for which we propose the provisional name Nijmegen Breakage Syndrome Because of its main characteristics viz chromosome instability, microcephaly, stunted growth mental retardation, skin abnormalities and immunodeficiency the new syndrome fits very well into the series of the classical chromosome breakage disorders (Table 4)

Four of the patients' phenotypically normal family members (the father and three of the four siblings still alive) each had one cell with a typical translocation We think this could be an expression of a heterozygotic state This would then be comparable to the situation in ataxia telangiectasia where a low percentage of cells with marker translocations has been found in a number of obligate carriers (21) Similar marker translocations have been found though in very low frequencies in the blood of apparently normal individuals (27, 28 29) We are inclined to believe that at least a number of the phenotypically normal individuals with such marker translocations are heterozygous carriers of some chromosomal instability disease such as ataxia telangiectasia (22) or the one in our family

If heterozygosity for the present disorder can indeed be detected cytogenetically, chromosomal studies of healthy family members might be of help in assessing their genetic risk Carrier-detection in our family might perhaps also be of value in the recognition of cancer-prone individuals, since it is known that not only the homozygous patients but possibly also the heterozygous carriers of chromosomal instability diseases have a predisposition to develop neoplasia (30, 31) It has for instance been estimated that 5% of all persons dying of malignancy below the age of 45 may carry an ataxia telangiectasia gene (30) Fortunately at the present time there appears to be no predisposition towards malignancy in the family studied but regular follow up studies of all family members will be carried out

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HYPERSENSITIVITY TO IONIZING RADIATION, IN VITRO, IN A NEW CHROMOSOMAL BREAKAGE DISORDER, THE NIJMEGEN BREAKAGE SYNDROME

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Hypersensitivity to ionizing radiation, in vitro, in a new chromosomal breakage disorder, the Nijmegen Breakage Syndrome

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Summary

The Nijmegen Breakage Syndrome (NBS) is a new chromosomal instability disorder different from ataxia telangiectasia (AT) and other chromosome-breakage syndromes. Cells from an NBS patient appeared hypersensitive to X-irradiation X-rays induced significantly more chromosomal damage in NBS lymphocytes and fibroblasts than in normal cells. The difference was most pronounced after irradiation in G_2. Further, NBS fibroblasts were more readily killed by X-rays than normal fibroblasts. In addition, the DNA synthesis in NBS cells was more resistant to X-rays and bleomycin than that in normal cells. The reaction of NBS cells to X-rays and bleomycin was similar to that of cells from patients with ataxia telangiectasia

Our results indicate that NBS and AT, which also have similar chromosomal characteristics, must be closely related.

In two earlier reports, we have described spontaneous chromosomal instability and multiple abnormalities of chromosomes Nos. 7 and 14 in a 12-year-old boy with a hereditary disorder clinically characterized by small size, microcephaly, mental retardation, 'café-au-lait' spots on the skin and serious immunodeficiencies (Hustinx et al., 1979; Weemaes et al., 1981). The cytogenetic abnormalities, and a number of other features present in the patient and in an older brother strongly resembled those found in the classical chromosomal breakage disorder ataxia telangiectasia (AT) (Boder, 1975, Aurias et al., 1980; Scheres et al., 1980). However, the diagnosis ataxia telangiectasia could not be made in our patients because they had neither

ataxia nor oculocutaneous telangiectasia In all variants of AT hitherto known ataxia is already present in early childhood and oculocutaneous telangiectasias are usually first observed before the age of 7 (Boder, 1975) We therefore strongly suggest that we have detected a new chromosome-breakage disorder for which we propose the provisional name Nijmegen Breakage Syndrome (Weemaes et al , 1981)

Patients with AT are unusually sensitive to X-rays, which was first noticed after the severe effects of standard therapeutic X-irradiation (Gotoff et al , 1967, Morgan et al , 1968, Cunliffe et al , 1975) The X-ray hypersensitivity is also evident in vitro when compared with normal cells, irradiated AT cells in culture consistently show an increased lethality (Taylor et al , 1975, Cox et al , 1978, Paterson and Smith, 1979), elevated levels of chromosomal aberrations (Higurashi and Cohen, 1973, Taylor, 1978, Natarajan and Meyers, 1979) and a relatively radioresistant DNA synthesis (Houldsworth and Lavin, 1980, Edwards and Taylor, 1980, Painter and Young, 1980, de Wit et al , 1981) Because of the cytogenetic similarities between AT and NBS, we decided to study the effect of X-rays on the colony-forming ability, the chromosomes and the rate of DNA synthesis in cells from the NBS patient In addition we measured their capacity for DNA-repair synthesis

Our data indicate that the radiation responses of the cells from the NBS patient are similar to those seen in AT cells

Materials and methods

Effect of X-rays on chromosomes

For the cytogenetic experiments, peripheral blood lymphocytes and fibroblasts were used In the lymphocyte studies, samples of freshly drawn whole blood were irradiated directly after venipuncture (lymphocytes in G_0) and then cultured for 42 h in RPMI 1640 medium containing 0 01% PHA as a mitogen and supplemented with 20% foetal calf serum (FCS) 2 h before the harvesting, colchicine was added to a final concentration of 0 05% Chromosome preparations were made according to Scheres (1976), stained with Giemsa and scored for aberrations Chromosomal damage was also scored after irradiation of the lymphocytes in the S or G_2 phase of the cell cycle Lymphocytes from the NBS patient and from a normal volunteer were first cultured for 48 h, followed by addition of thymidine to a final concentration of 0.3 mg/ml, to produce some synchronization of the cells by inhibiting their DNA synthesis The block was released after 17 h by washing the cells twice with RPMI 1640 They were then resuspended in fresh RPMI with FCS, exposed to X-rays cultured for a further period of 6 h and harvested for chromosome analysis

In the fibroblast studies, freshly seeded cells from the NBS patient, 2 AT patients and a normal individual were grown for 1 day in Ham's F10 with 20% FCS, and then irradiated. The cultures were incubated for a further period of 20 h, colchicine was added and chromosome preparations were made in the usual way

For comparison of the chromosomal radiosensitivity of the various cell lines, the numbers of dicentric chromosomes, acentric fragments and chromatid breaks per 100 cells were determined

Survival experiments

Cellular survival after exposure to X-rays was measured with the thin-feeder-layer technique of Cox and Masson (1974) Exponentially growing fibroblast cultures were trypsinized, treated with X-rays and seeded on dishes with feeder cells prepared on the day before After being cultured for 2 weeks, the cells were fixed and stained. and the number of colonies with at least 50 cells was scored on triplicate dishes D_0 values were calculated from the slope of the linear part of the semi-logarithmic plots of survival against X-ray dose

Inhibition of DNA synthesis

The effect of X-rays on the rate of DNA replication in fibroblasts and PHA-stimulated lymphocytes was measured as described earlier (de Wit et al., 1981, Jaspers et al , 1981) Briefly, the cells were prelabelled with [^{14}C]thymidine, exposed to X-rays and then pulse-labelled with [^{3}H]thymidine. Incubation was stopped in ice-cold phosphate-buffered saline Cells were harvested by scraping The ratio of ^{3}H to ^{14}C radioactivities in trichloroacetic acid precipitates was taken as a measure of the rate of DNA replication.

DNA repair synthesis

Repair replication was measured, after high doses of X-rays, by buoyant-density centrifugation of the DNA in NaI gradients as described by Paterson et al. (1976). The DNA content was measured with the fluorescent dye Hoechst 33258, according to Labarca and Paigen (1980). During irradiation the pH was checked with 20 mM Hepes (pH 7 4) in the medium

Conditions of irradiation

X-rays (300 kV) were delivered at room temperature under conditions of atmospheric oxygen pressure In the cytogenetic experiments, the X-rays were generated by a Siemens Stabilipan X-ray machine operating at 12 mA and providing a dose rate 50 rad min^{-1}. (100 rad = 1 Gy) In the other experiments, a Philips machine was used (10 mA, 175 rad min^{-1}).

Results

Chromosomal aberrations induced by X-rays

Table 1 lists the results of the cytogenetic experiments with the fibroblasts X-rays induced both chromosomal and chromatid aberrations. The NBS, AT5BI and AT2NM cells were more sensitive to the chromosome-damaging influence of X-rays than were normal cells. The numbers of dicentrics, breaks and acentric fragments per cell were significantly higher in the NBS and AT cell lines than in the normal cultures.

In the lymphocyte experiments the NBS cells appeared more radiosensitive than normal cells (Table 2). The differences between the NBS and the normal lymphocytes were dependent upon the phase in which the cells had been irradiated. In

TABLE I

FREQUENCIES OF CHROMOSOMAL ABERRATIONS INDUCED IN NORMAL, AT AND NBS FIBROBLASTS BY X-IRRADIATION

The numbers in italics represent the NBS and AT values that differed significantly ($P < 0.001$, χ^2 test) from the values in normal cells (The dicentric frequency in the unirradiated normal cells (3/100) might seem rather high, but it remained so after repeated testing)

	Dose	No scored cells	Aberrations/100 cells		
			Dicentrics	Acentric fragments	Chromatid breaks
Normal	Control	200	3	0	4
	150 rad	400	6	72	13
AT5BI	Control	100	3	10	8
	150 rad	348	*18*	*143*	*24*
AT2NM	Control	200	0	11	13
	150 rad	200	*15*	*161*	*39*
NBS	Control	123	3	19	9
	150 rad	405	*18*	*112*	*25*

TABLE 2

FREQUENCIES OF CHROMOSOMAL ABERRATIONS IN BLOOD LYMPHOCYTES AFTER X-IRRADIATION

The numbers in italics represent the NBS values that differed significantly from the values in the normal lymphocytes ($P < 0.001$, χ^2 test).

		Dose	No. scored cells	Aberrations/100 cells		
				Dicentrics	Acentric fragments	Chromatid breaks
G_0	Normal	Control	42	0	0	2
		100 rad	51	18	31	0
	NBS	Control	96	0	1	5
		100 rad	50	22	51	4
G_2/S						
Expt. I	Normal	Control	42	0	0	2
		100 rad	25	0	32	20
	NBS	Control	96	0	1	5
		100 rad	25	0	56	*172*
Expt II	Normal	Control	42	0	0	2
		100 rad	51	0	0	18
	NBS	Control	96	0	1	5
		100 rad	50	2	18	*144*

G_0-irradiated lymphocytes the most frequently observed aberrations were of the chromosome type (dicentrics and acentrics), the level of which was about 1 5 times higher in NBS than in normal lymphocytes (1 2 times for the dicentrics and 1 7 times for the acentric fragments) The S/G_2-irradiated lymphocytes contained mainly chromatid-type lesions, the level of which was about 8 times higher in NBS than in the control lymphocytes

Cell survival after X-irradiation

The colony-forming abilities of NBS, AT and control fibroblasts after exposure to various doses of X-rays are given in Fig 1 Both the NBS and AT5BI cells were killed by X-rays more readily than were normal fibroblasts The NBS cells showed a radiosensitivity intermediate between those of normal and the AT5BI cells The mean D_0 values were 47 rad for AT5BI, 70 rad for the NBS, and 130 and 145 rad for the normal fibroblast strains.

DNA replication after X-irradiation

The rates of DNA synthesis after exposure to X-rays in NBS, AT and normal cells are presented in Figs 2 and 3 Both in cultured fibroblasts and in PHA-stimulated lymphocytes from the NBS patient, DNA replication was inhibited to a significantly lesser extent by X-rays than in the normal cells ($p < 0\ 02$, Mann–Whitney U test) after exposure to doses up to 4000 rad The relative rates of DNA synthesis were comparable in irradiated NBS and AT cells

Kinetic studies of the DNA synthesis in lymphocytes after X-irradiation revealed

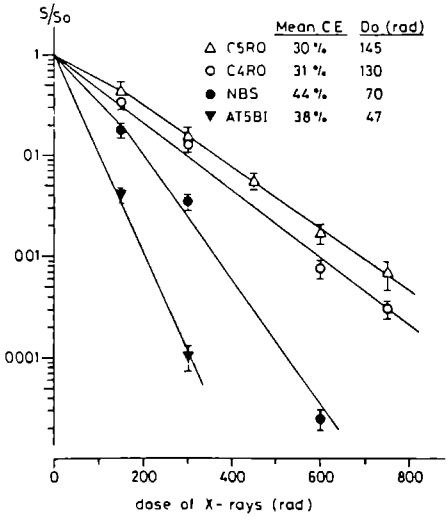
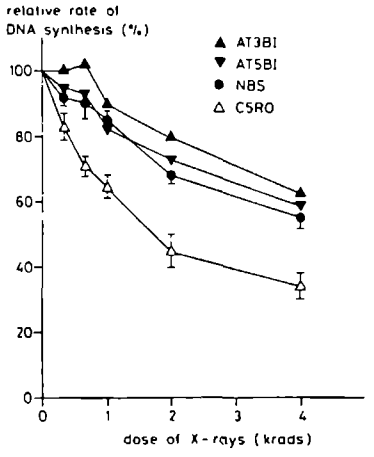


Fig 1 Cellular survival of cultured fibroblasts after exposure to X-rays In each experiment triplicate dishes were scored at every dose The D_0 values were calculated from the linear portion of the plots by regression analysis Open symbols represent normal cells (C5RO, 2 Expts and C4RO, 3 Expts) AT5BI 1 Expt and NBS cells, 3 Expts



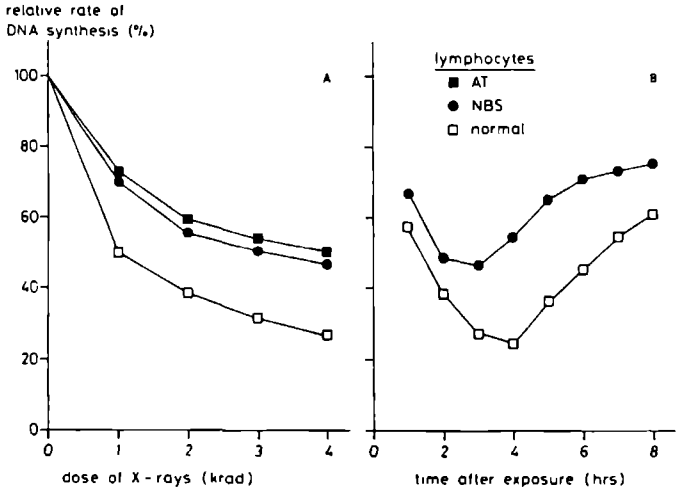


Fig. 2. Inhibition of DNA synthesis in cultured fibroblasts after exposure to X-rays. The rate of DNA replication was measured during the first 4 h after irradiation. Data in each experiment are means of at least 2 dishes. When there were more than 2 Expts., bars indicate the standard error. Normal C5RO cells, 5 Expts.; AT3BI and AT5BI, 2 Expts.; cell strain NBS cells, 3 Expts.

Fig. 3. Inhibition of DNA synthesis in PHA-stimulated lymphocytes after exposure to X-rays. Panel A: dose–response relationship. Experimental procedure was the same as in Fig 2. Panel B: kinetics of DNA replication. Cells were exposed to 2 krad of X-rays and pulse-labelled for 60 min with ^{3}H-TdR at different times after exposure. The abscissa in B indicates the time of harvesting All data are means of duplicate determinations, carried out in a single experiment. Duplicates always matched within 5%.

that, in both NBS and normal cells, there was an initial decrease in the rate of replication followed by a gradual recovery starting at about 3–4 h after irradiation (Fig. 3). At all times after exposure, the relative rate of DNA replication was higher in NBS than in normal cells. This time course was the same as that observed in AT fibroblasts earlier (Jaspers et al., 1982).

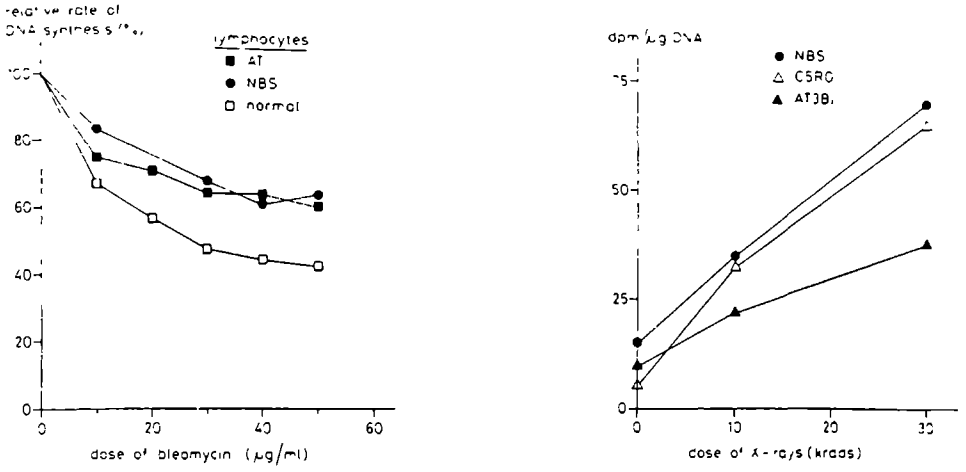


Fig. 4. Inhibition of DNA synthesis in PHA-stimulated lymphocytes after exposure to bleomycin. Cells were treated with the indicated concentrations of the drug for 60 min and labelled for 4 h with ^{3}H-TdR. All data are means of duplicate determinations carried out in the same experiment as that given in Fig. 3.

Fig. 5. Repair replication induced by X-rays in cultured fibroblasts. The radioactivities incorporated by replicative DNA synthesis and by repair DNA synthesis were separated by a single banding in NaI-density gradients. Data are means of duplicate dishes.

A pattern similar to that seen after X-ray exposure was evident after treatment of PHA-stimulated lymphocytes with the radiomimetic drug bleomycin. Again the inhibition of DNA synthesis was less in AT and NBS than in normal cells (Fig. 4).

DNA repair synthesis

The DNA-repair capacity of the NBS fibroblasts was compared with that of normal and AT3BI fibroblasts. The latter cell line is known to be an excision-repair-deficient mutant (Paterson et al., 1976). The results are given in Fig. 5. The ability of the NBS cells to carry out radiation-induced repair DNA synthesis under the conditions used did not differ from that of normal cells.

Discussion

One of the most interesting features of the classical chromosome-breakage syndromes is the hypersensitivity of the cells to DNA-damaging agents (see e.g. Arlett and Lehmann, 1978). The experiments described in this paper show that the newly detected breakage syndrome NBS follows this rule: the NBS cells are typically hypersensitive to X-rays. This was established in three experimental approaches, as follows: X-rays induced significantly more chromosomal damage in NBS cells than in normal cells; NBS fibroblasts were more readily killed by X-rays than normal fibroblasts; DNA synthesis in NBS cells was more resistant to X-rays and bleomycin than that of normal cells.

The patterns of chromosomal damage induced by X-rays in NBS and AT lymphocytes seem to depend in a similar way on the phase in which the cells have been irradiated. Irradiation of NBS cells in G_0 resulted in aberrations of the chromosomal type, the level of which was only 1.5 times higher than in normal lymphocytes. The difference between NBS and normal cells was greater when the cells had been irradiated in G_2: as compared with the normal, the G_2-irradiated NBS lymphocytes contained 8 times more chromatid breaks. These data parallel those obtained by Taylor (1978) and by Natarajan and Meyers (1979) in their studies of the radiosensitivity of AT cells at different stages of the cell cycle.

The survival experiments also showed conformity of the NBS and AT cells. Both were more readily killed by X-rays than normal cells. The D_0 values obtained with the cell strain AT5BI and the normal cells agreed well with those obtained in various other laboratories (Cox et al., 1978; Weichselbaum et al., 1980; Arlett and Harcourt, 1980). The D_0 value of 70 rad seen in the NBS cell strain was significantly different from the normal range and within the range obtained with the cells of at least 12 AT patients in the studies mentioned above.

Another cellular characteristic that was similar in NBS and AT was the rate of DNA synthesis. Its inhibition by X-rays was less pronounced than that in normal cells. In the lymphocyte as well as in the fibroblast experiments, the dose–response curves for the NBS and AT cells matched closely (Figs. 2 and 3), and the kinetics of DNA synthesis in NBS cells were much like those in AT cells, as reported earlier (Jaspers et al., 1982).

AT cells are hypersensitive not only to X-rays, but also to certain chemical agents such as bleomycin (Taylor et al., 1979; Lehmann and Stevens, 1979). The inhibition of DNA synthesis by bleomycin in PHA-stimulated NBS lymphocytes was diminished as compared with normal controls. This result is in line with data obtained on AT fibroblasts after exposure to this agent (Cramer and Painter, 1981; Jaspers et al., 1982). Also, preliminary cytogenetic and cell survival experiments have shown that NBS cells are as sensitive as AT cells to this drug (unpublished results).

Summarizing our data, we conclude that NBS and AT cells are similar in their unusual response to ionizing radiation and bleomycin.

Any definite conclusion about the primary defect in the NBS cells will be highly speculative at present, but the resemblance of NBS and AT in their cellular characteristics and the occurrence of the same typical translocations in chromosomes Nos. 7 and 14 (Weemaes et al., 1981) suggest that the basic defects leading to their peculiar X-ray responses are also closely related. However, AT is a heterogeneous disease. Variability in the clinical symptoms has been observed by several authors (see e.g. Jason and Gelfand, 1979; Kraemer, 1977). Biochemically, AT cell strains behave differently as well: some are defective in performing repair DNA synthesis of radiogenic DNA damage whereas other strains are normal in that respect (Paterson and Smith, 1979). NBS cells are similar to the latter category of AT cell strains regarding their normal level of DNA repair synthesis after high doses of X-rays. Finally, work of Painter and Young (1980) and more recent data obtained by Jaspers and Bootsma (1982) have demonstrated extensive genetic heterogeneity in AT. Using the diminished inhibition of DNA replication after X-ray exposure as a

marker, they discerned 4 different genetic complementation groups. Since, in cells from the patients with NBS, we found a defect in the regulation of DNA replication similar to that in AT cells, complementation studies after fusion of NBS cells with different AT cell strains are possible. This analysis should resolve the question whether the NBS represents a distinct genetic entity or is rather a reflection of phenotypic variation within a known AT complementation group.

Acknowledgements

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Chapter 4

IMMUNOLOGICAL AND CYTOGENETIC STUDIES IN ATAXIA TELANGIECTASIA

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SUMMARY

Immunological and cytogenetic studies were performed in six patients with ataxia telangiectasia (AT). Immunological disturbances were found in these patients: immunoglobulin deficiencies (IgA, IgE, IgG2 and IgG4), decreased cellular immunity and a defect in the synthesis of specific antibodies. Cytogenetic studies revealed chromosome 7 and/or 14 abnormalities in all patients. X-irradiation of AT cells induced an excessive increase in chromosome- and chromatid- breaks. The DNA synthesis after X-irradiation was less reduced in cells from AT patients than in cells from normal controls. The possibilities of an early diagnosis have been outlined and in addition, the possible relationship between the cytogenetic and the immunological anomalies has been discussed.

INTRODUCTION

Ataxia telangiectasia (AT) is an autosomal recessive hereditary syndrome in which the most characteristic clinical symptoms are progressive cerebellar ataxia, progressive oculocutaneous telangiectasias and recurrent sinopulmonary infections. There are distinct immunological disturbances which vary from patient to patient and can aggravate with age. The most frequent immunological disturbances are IgA deficiency (70%), IgE deficiency (80%), and defect in the cellular immunity (Stiehm and Fulginiti, 1980). Cytogenetic studies may show a typical chromosome instability in both cultured lymphocytes and

fibroblasts. Especially specific sites in the chromosomes 7 and 14 are involved in this instability (Aurias et al, 1980; Scheres et al, 1980).

AT patients with a malignancy, who have been submitted to standard X-ray therapy, showed very serious damage in and around the irradiated field (Gotoff et al, 1967; Morgan et al, 1968). Hypersensitivity to X-rays also appeared in cultured cells of AT patients (Taylor et al, 1975). X-irradiation leads to an increase of the percentage of cells with an abnormal chromosome pattern and also to a disturbed DNA synthesis as compared to that in normal cells. The syndrome belongs to the socalled chromosome instability syndromes and shares several features with these disorders: skin abnormalities as cafe au lait spots and vitiligo spots, postnatal growth retardation, enhanced sensitivity for specific mutagenic factors and a high tendency towards malignancies (Hecht and Kaiser McCaw, 1977; Hustinx et al, 1977 ; Weemaes et al, 1981).

Chromosome instability syndromes as Bloom's syndrome , Fanconi's anemia, Nijmegen breakage syndrome and ataxia telangiectasia are already for more then a decade, one of our main interests. During the recent years we have investigated 12 patients with AT. In six of them extensive studies could be done. In the present paper the most important results of these studies are presented. The possibilities of early diagnosis will be outlined, and in addition, the possible relationship between the cytogenetic and the immunological anomalies will be discussed.

CLINICAL FINDINGS

Table 1 surveys some of the clinical data pertaining to our patients. The ataxia became manifest when the children began to sit and walk. The oldest cases (Nrs. 1,2,3 and 4) were confined to a wheelchair at the age of 10 years. The age at which the telangiectasias became apparent was variable. Two of our patients have already deceased: patient two at the age of 14 years from respiratory insufficiency and patient 4 at the age of 9 years from a cytomegalovirus infection which appeared when the patient was treated with prednison because of a thrombocytopenia.

IMMUNOLOGICAL INVESTIGATIONS

a. Humoral immunity

In three of the six patients an IgA deficiency was detected (Table 2). Within a family patients with and without IgA were found (Nrs.2 and 3, and Nrs. 4 and 5). In IgA-deficient patients anti-IgA antibodies can originate. Such antibodies could be demonstrated in patients 2 and 4 at the age of 10 and 9 years respectively, but till now not in patient 6. Patient 1 showed a markedly decreased serum IgA concentration (2 IU/ml); 8 years earlier the serum IgA concentration was 16 IU/ml, i.e. there has been a conspicuous decrease of the serum IgA. In 5 of the six patients an IgE deficiency was demonstrated (Table 2). Patient 2 has had a normal IgE level at the age of 10 and 12 years, but later he also became IgE deficient. The serum IgD levels were were normal in all patients. The serum IgG concentrations varied

Patient number	Year of birth	Sex	Onset of ataxia (years)	Onset of telangiectasia (years)	Age at diagnosis (years)	Recurrent respiratory infections	Cafe au lait spots	Mental retardation	Growth retardation
1	1962	F	2	?	9	+	+	+	+
2	1964	M	2	4	8	+	+	-	-
3	1972	M	2	3	2	-	+	-	+
4	1971	M	2	6	7	+	+	-	-
5	1979	F	2	3	2	-	+	-	-
6	1977	F	2	6	5	+	+	-	+

TABLE 1. Clinical data of the patients
The patients 2 and 3, as well as the patients 4 and 5 are sibs.

Patient number	Age (years)	IgM	IgG total	IgG1	IgG2	IgG3	IgG4	IgA	IgD	IgE
1	18	161	215	240	<5	10**	428	2**	132	<0.5
2	12	94	66*	85	<5	126	<5	<1		59
3	8	110	43*	59	<5	43	<5	105	65	1
4	9	201	168	130	<5	77	<5	<1	70	<0.5
5	4	272	45	67	<10	82	<10	67	29	<0.5
6	5	79	94	134	<10	33	<10	<1	109	<0.5

TABLE 2. Serum immunoglobulins (expressed in IU/ml) and IgG subclasses (expressed as percentage of normal standard serum).
* Level slightly decreased
** Level markedly decreased
(For normal values see Weemaes et al, 1979 and van der Giessen et al, 1975).

between normal and slightly decreased. The investigation of the IgG subclasses revealed an IgG2 deficiency in all 6 patients, and an IgG4 deficiency in four of them (Table 2).

In all patients the percentage of B cells among peripheral blood lymphocytes appeared to be normal. In the patients with IgA deficiency no surface-IgA-positive B cells were found.

In all patients a good synthesis of specific antibodies was observed after administration of diphteria-, tetanus- and polio-vaccine booster. However, the primary response to haemocyanine vaccine was abnormal in the five cases tested. In three of them only a slight IgM response was found. In none of them IgA or IgG antibodies against haemocyanine were detected (Weemaes et al, 1984).

b. Cellular immunity

The absolute number of T cells was normal in only 2 of the 6 patients (Nrs. 1 and 2). The in vitro response of the peripheral blood lymphocytes to the mitogens phytohemagglutinin and pokeweed mitogen was low in all patients. Longitudinal studies revealed that this response was not constant for a given patient and that it could vary from low to markedly depressed. In three of the 6 patients a positive in vitro reaction has been found to stimulation with tetanus toxoid. There was none or only minimal response to other antigens (diphteria toxoid, haemocyanin).

a. Spontaneous chromosome aberrations

The karyotype of all patients was basically normal (46,XY or 46,XX). The study of 100 metaphases of AT patients and controls has revealed more chromatid- and chromosome breaks in the cells

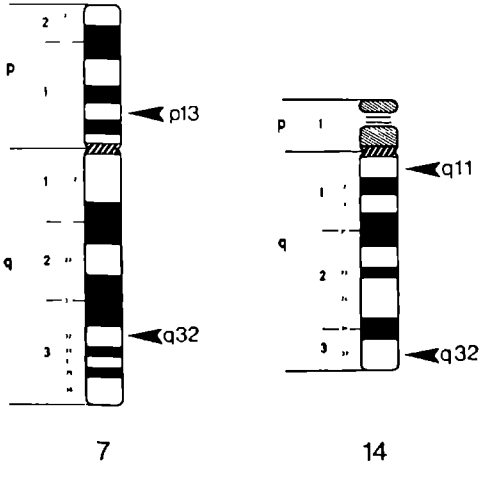

FIGURE 1a.
The exchange points on the chromosomes 7 and 14, which are frequently involved in the rearrangements in cultured lymphocytes of AT patients.

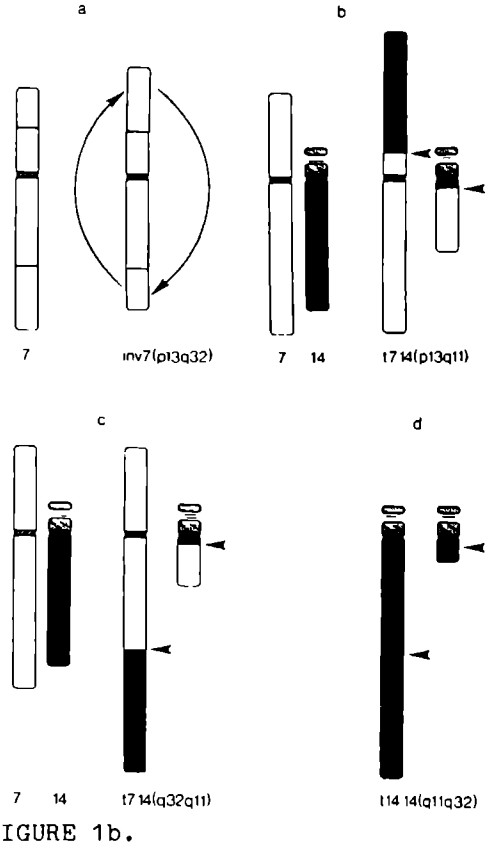

FIGURE 1b.
The most frequent chromosome 7 and 14 translocations that are found in cultured lymphocytes of AT patients (cf Table 3).
a. inv(7)(p13;q32)
b. t(7;14)(p13;q11)
c. t(7;14)(q32;q11)
d. t(14;14)(q11;q32)

of the patients than in those of the controls. In addition, in all patients chromosome anomalies were observed, which are very

	our patients							
	1	2	3	4	5	6	total	Aurias et al, 1980
Total number of cells	47	147	249	88	49	80	660	927
t(7;14)(q32;q11)	-	-	-	-	-	2	2	8
t(7;14)(p13;q11)	-	1	1	1	-	-	3	8
t(7;7)(p13;q32)	-	-	-	-	-	-	-	3
inv(7)(p13;q32)	-	-	2	3	2	2	9	29
t(14;14)(q11;q32)	45	-	-	-	-	-	45	2
inv(14)(q11;qter)	-	-	-	-	-	-	-	6
Complex (7;7)	-	1	-	-	-	-	1	3
Complex (14;14)	-	-	-	-	-	-	-	3
Other rearrangements of chromosome 7 and 14	-	2	-	1	-	-	3	14
Rearrangements of other chromosomes	2	2	2	10	1	-	17	82
Rearrangements of unspecified chromosomes	1	2	7	5	-	-	15	-
Mean number of chromosome anomalies per cell							0.14	0.17

TABLE 3. Survey of the chromosome abnormalities in our 6 patients. The last column gives the results of the cytogenetic studies by Aurias et al, 1980 in 11 AT patients.

characteristic for AT. The most frequent abnormalities are reciprocal translocations of the chromosomes 7 and 14 (Table 3). The exchange points involved in these translocations are localized at four sites: two in chromosome 7 (7p13 and 7q32) and two in chromosome 14 (14q11 and 14q32) (Figs. 1). Generally the percentage of cells with such an abnormality was low, but in one case it was even more than 90% (Table 3).

Induced chromosome aberrations

In AT both in vivo and in vitro hypersensitivity to X-irradiation has been reported (Taylor et al, 1975; Hecht and Kaiser McCaw, 1977). The cells of our patients 1, 3, 4, 5 and 6 have been

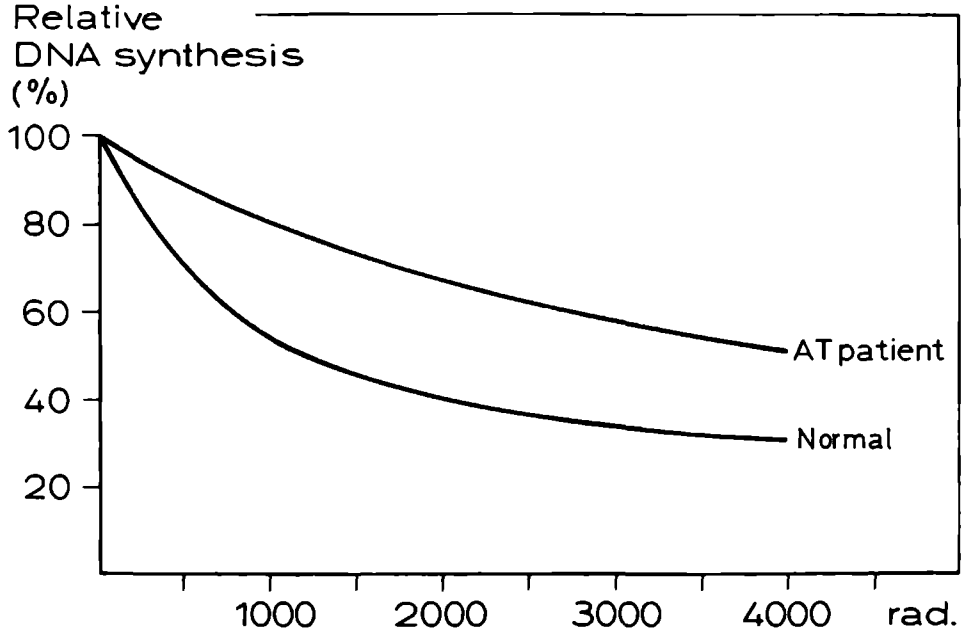

FIGURE 2.
The inhibition of the DNA synthesis in PHA stimulated lymphocytes after X-irradiation (patient 6). The DNA synthesis is measured during the first 4 hours after X-irradiation. The inhibition of the DNA synthesis in the cells of AT patients is significantly less than in the cells of normal controls.

tested for this X-ray hypersensitivity. Depending on the experimental conditions 1.5 to 10 times as much chromatid- and chromosome aberrations were found in their X-ray treated (1 Gy) PHA stimulated lymphocytes and fibroblasts as in normal cells.

INHIBITION OF THE DNA-SYNTHESIS AFTER X-RAY IRRADIATION

The hypersensitivity to X-rays manifests itself at the cellular level also otherwise. The survival of AT cells after X-irradiation is clearly less than normal. Moreover, the DNA synthesis in cultured AT lymphocytes or fibroblasts appears to be less reduced by X-irradiation than hte DNA synthesis of normal cells (Jaspers et al, 1981; Taalman et al, 1983). The inhibition of the DNA synthesis after X-irradiation in PHA stimulated lymphocytes was investigated in the patients 3 and 6. This inhibition turned out to be less than in cells of normal controls (Fig. 2).

DISCUSSION

The 6 patients described in this paper meet all the clinical criteria of AT (Stiehm et al, 1980). Mostly the diagnosis of AT is made late i.e. when both cerebellar ataxia and telangiectasias are manifest. If in a family there is already an AT patient , the diagnosis in the children who follow can clinically be made when the ataxia is present , usually in the second year (patients 3 and 5; Table 1).

In atactic patients under the age of 6 years who do not have telangiectasias immunological screening cannot be of help for the exclusion of the diagnosis AT: the levels of the immunoglobulins, the number of T lymphocytes and the results of the in vitro lymphocyte stimulation tests are too variable.

The cytogenetic studies revealed in all patients structural chromosome abnormalities in a number of their cultured lymphocytes. The frequency and the diversity of the rearrangements in our patients are comparable to those reported by Aurias et al (1980)(Table 3). The chromosomes 7 and 14 are preferentially involved in these rearrangements. For making early diagnosis this type of cytogenetic studies is not sufficient, as the frequency of the typical rearrangements can be very low especially at young age. The investigation of chromosome aberrations induced by X-irradiation seems to be more promising for early diagnosis, but such studies are very time consuming.

For early diagnosis the investigation of the DNA synthesis might perhaps offer the most reliable possibility. It has been found both in our patients in in those from other authors (Jaspers et al, 1981) that the inhibition of the DNA synthesis after X-irradiation of the cells is less than in controls. It is assumed that this abnormal reaction represents a basic defect in the control system regulating repair and replication of the chromatin or the DNA matrix (Painter and Young, 1980; de Wit et al, 1981). Such a basic defect will be measurable already in very early phases of life, even before the presence of ataxia or telangiectasias. In patient 6 in whom the diagnosis AT was presumed because of her ataxia and IgA deficiency, this test appeared decisive already one year before the telangiectasias

became manifest. This rather simple test offers perspectives for early diagnosis and might also be of significance for prenatal diagnosis (Jaspers et al, 1980).

In vitro hypersensitivity of the chromosomes for X-rays was established in all our patients. Moreover it is known that radiotherapy in AT patients can lead to severe reactions (Gotoff et al, 1967;Morgan et al, 1968). Therefore, it is important to minimize diagnostic and therapeutic X-irradiation of patients with AT.

Immunological disturbances do occur in all patients, but they do not lead to an abnormal course of childhood diseases as chickenpox or to serious infections. However, they might contribute to the development of bronchiectasias, respiratory insufficiency and malignancies (often of the lymphoreticular system).

Two patients with IgA deficiency (Nrs.2 and 4) appeared to have anti-IgA antibodies at the age of circa 10 years. In children of that age with selective IgA deficiency it is exceptional that anti-IgA antibodies can be detected (own observation). The presence of anti-IgA antibodies is a contra-indication for treatment with gammaglobulin or plasmatransfusions sometimes taken into consideration in case of defects in the humoral immunity.

One might wonder what the relationship is between the presence of the immunological disturbances and the typical involvement of the chromosomes 7 and 14. Pertaining to this it is noteworthy that the genes coding for the immunoglobulin heavy chains are localized on chromosome 14 in band q32 (McKusick, 1983). In one of our patients (Nr.1) with a IgA deficiency a tandem

translocations (14;14)(q11;q32) was found in more than 90% of her cultured lymphocytes. Possibly there is a relation between the IgA deficiency and the involvement of 14q32. However, also the other patients have imunoglobulin deficiencies (Table 2), but no translocation (14;14) was present in their cells. It is unknown if the other sites involved in the rearrangements that are frequently seen in our patients - 7p13, 7q32 and 14q11 - contain loci or genes with a coding or regulating role in the synthesis of immunoglobulins. It is unlikely that the AT gene itself is directly responsible for the origin of the immunoglobulin deficiency, as within one family both patients with and without IgA occur (Table 2). Perhaps it would be possible to detect more clones with a translocation (14;14) when cytogenetic studies are done on lymphocytes cultured after stimulation with a specific B-cell mitogen. In the future recombinant DNA techniques might perhaps provide better possibility for the study of a relationship between chromosomal aberrations and immunological disturbances.

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Chapter 5

VARIANTS OF ATAXIA TELANGIECTASIA OR NEW CHROMOSOME INSTABILITY SYNDROME?

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VARIANTS OF ATAXIA TELANGIECTASIA OR NEW CHROMOSOME BREAKAGE SYNDROME?

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Ataxia telangiectasia (AT) is an autosomal recessive disorder characterized by progressive cerebellar ataxia, oculocutaneous telangiectasia and recurrent sinopulmonary infections. Pulmonary insufficiency is the most frequent cause of death. Patients have a predisposition to the development of neoplasia and many patients develop cancer during the first two decades of life.

Both humoral and cellular immunity are abnormal, but show considerable inter-patient variation. IgA, IgE and IgG subclass deficiencies are common (1-4). Antibody synthesis is disturbed (5-7). The number of T cells is decreased in most patients (8) and the in vitro response of peripheral blood lymphocytes to stimulation with mitogens and antigens is frequently impaired (8,9).
AT belongs to the group of classical chromosome breakage syndromes. Cells of AT patients in vitro usually display an increased frequency of spontaneous chromosomal breaks and rearrangements, the latter consisting mainly of translocations and inversions with specific involvement of the chromosomes 7 and 14 at the sites 7p13, 7q32, 14q11 and 14q32 (10-12)(Fig.1.).

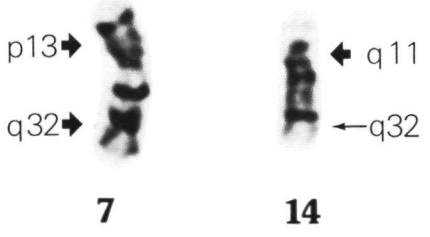

Fig. 1. The four "fragile sites" in the chromosomes 7 and 14 in Ataxia Telangiectasia and Nijmegen Breakage Syndrome.

AT cells are hypersensitive to ionizing radiation and after X-ray irradiation the chromosome and chromatid type of aberration are greatly increased (13) Also, the rate of DNA synthesis after exposure to X-rays is abnormal, the rate of DNA synthesis in cultured AT lymphocytes and skin fibroblasts being inhibited to a significant lesser extent than in cells from normal individuals (14)(Fig 2).

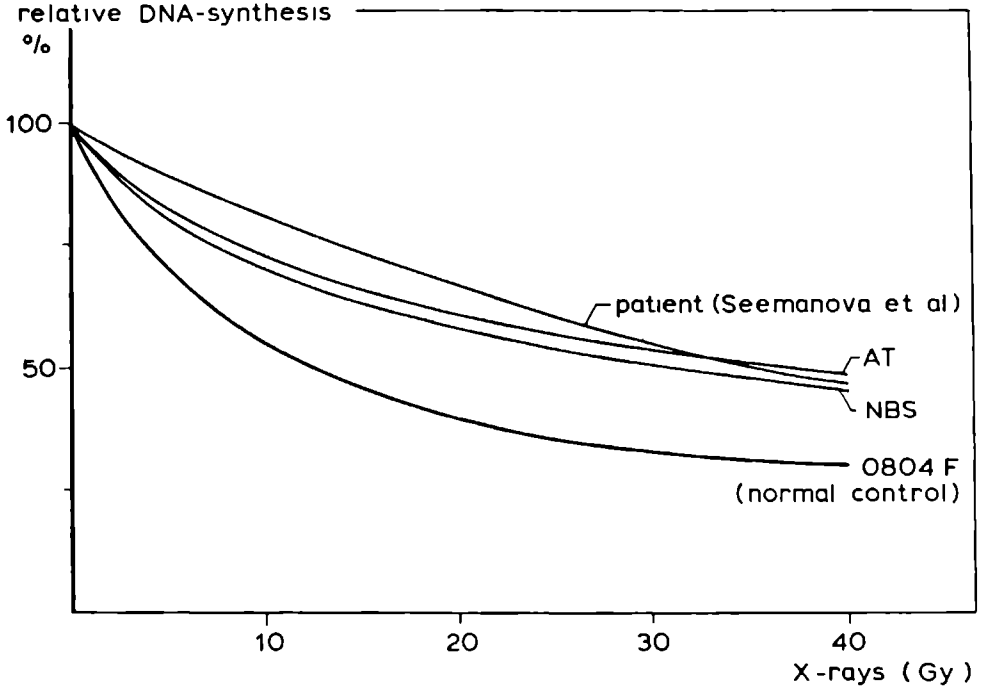

Fig. 2. Inhibition of DNA synthesis in PHA-stimulated lymphocytes after exposure to X-rays. The rate of DNA replication was measured during the first 4 hours after irradiation.

A distinct genetic heterogeneity has been demonstrated in classical AT, at least four complementation groups having been proposed to date (15).

However, the above mentioned immunological, cytogenetic and cell biological properties are not specific for AT. Recently, we investigated several patients with microcephaly having neither ataxia nor oculocutaneous telangiectasias but with immunological, cell biological and cytogenetic conspicuous resemblances to classical AT.

CLINICAL CONSIDERATIONS

In 1981 we reported two sibs with microcephaly, stunted growth, mental retardation, café-au-lait spots, immunodeficiency, and chromosome instability (16). The propositus, a boy HH, was born in 1969. His growth remained below P10 He was microcephalic from birth, his head circumference at 16 years was 48 cm (<P3). During infancy he suffered from repeated upper respiratory tract infections. In childhood he had some episodes of bronchitis and a pneumonia once. The course of chickenpox and measles was unremarkable. He is slightly mentally retarded. Neurological examination did not reveal further abnormalities.

An older boy (MH), born in 1964, showed similar clinical signs. He also was microcephalic (<P3), mentally retarded and of subnormal height (<P3). He suffered repeatedly from otitis media, pneumonia, and urinary tract infections. Hypoimmunoglobulinaemia was at the age of 4 years. Despite gammaglobulin treatment he sustained respiratory tract infections and died from respiratory failure at the age of 6 years.

In 1985 Seemanová et al (17) described six independent families with nine patients showing microcephaly, immunodeficiency, and increased risk for lymphoreticular malignancy These patients resemble each other clinically: a low and receding fore head, slightly upward slant of the palpebral fissures, and a receding mandible are present after the age of 2 years. The height was below the 3rd percentile in all-five female and two of the four male patients. Their psychomotoric development was reported to be normal. A striking predisposition to upper respiratory tract infections was noted in all patients. Four suffered from repeated bronchopneumonas and one died at the age of 6 months of pneumonia (17). Four of the five deceased patients died of a malignancy of lymphoreticular origin (17).

We had an opportunity to do supplementary investigation on the four patients still alive and to compare the results with those of our Dutch patients. Three of them (patient 2,7 and 8 of Seemanová et al) resembled our patients in several aspects of the in vitro studies described before, whereas the fourth (case 9) did not.

IMMUNOLOGICAL STUDIES

Quantitative serum immunoglobulin studies revealed low normal to normal serum IgM levels in all patients (table 1.). Serum IgG was deficient in two Czechoslovakian patients and greatly decreased in one Dutch boy (table 1.). IgA deficiency was established in both Dutch patients and in one Czechoslovakian girl. The two other patients had decreased serum IgA levels (table 1.). IgE and IgD were not detectable in three patients and greatly decreased in the fourth (table 1.).

TABLE I

IMMUNOLOGICAL, CYTOGENETIC AND CELL BIOLOGICAL DATA IN THE PATIENTS

	Dutch patients		Czechoslovakian patients		
	MH	HH	patient 2	7	8
Sex	male	male	female	male	female
Year of birth	1964	1969	1971	1981	1979
Age of death	6½ yr				
Immunological studies					
IgG IU/ml	34	109	5	2	53
IgA IU/ml	<1	<1	<2	9	28
IgM IU/ml	150	66	143	42	120
IgD IU/ml		<1	<1	<1	3
IgE IU/ml		<0,5	<0,5	<0,5	8
% T cells		↓to normal	↓	↓	↓
In vitro responses of lymphocytes to					
PHA		100%	↓		
PWM		50%			
antigens		low response			
Alpha fetoprotein		normal	normal	normal	normal
Cytogenetic studies					
7 and 14 rearrangements		present	present		present
sensitivity to X-rays		↑			↑
Cell biological studies					
DNA inhibition test					
in lymphocytes		abnormal	abnormal	abnormal	abnormal
in fibroblasts		abnormal	abnormal	abnormal	

In the Dutch patients antibody synthesis was disturbed (7). The lymphocytes of patient HH showed a normal in vitro response to PHA, but decreased to PWM (table 1). In addition, the percentage of E-rosette forming cells of HH proved to be normal, whereas the percentage of T_3 positive cells was decreased.

In the Czechoslovakian patients T cells were reported to be decreased and lymphocyte responses to PHA impaired (17)

In four patients the levels of alpha fetoprotein (AFP) were normal (table 1)

CYTOGENETIC AND CELL BIOLOGICAL STUDIES

In the surviving Dutch patient multiple rearrangements of chromosomes 7 and 14 were found. Most of the rearrangements were reciprocal translocations with breakpoints almost exclusively located at four sites. 7p13, 7q32, 14q11

and 14q32. These are the same sites that are involved preferentially in the rearrangements in classical AT (fig.1)(16). In the lymphocytes of the Czechoslovakian patients the same 7/14 rearrangements were detected (table 1). The lymphocytes of the third patient failed to grow sufficiently for detailed analysis.

After X-irradiation of lymphocytes an increase of the chromosome and chromatid aberations was found in the Dutch patient and one of the Czechoslovakian patients (table 1).

DNA synthesis in cultured lymphocytes of all four patients was impaired less by X-irradiation than in normal controls (fig.2). In fibroblasts of the three patients DNA synthesis after X-irradiation was also abnormal (table 1).

DISCUSSION

The clinical features and the laboratory findings of our and Seemanová's patients have a remarkable similarity: microcephaly, stunted growth, repeated respiratory tract infections, immunodeficiency, chromosomal instability with multiple 7/14 rearrangements, and X-ray hypersensitivity. Complementation analysis revealed that the Dutch patient did not belong to the AT complementation groups A,C and D (Taalman et al., to be published; Jaspers et al., to be published). No complementation occurred after fusion of the fibroblasts of the Dutch patient with fibroblasts of the Czechoslovakian patient 7 (Jaspers et al., to be published), which indicates that they might have the same basic genetic defect.

In 1981 we provisionally named the syndrome in our two patients:"Nijmegen Breakage Syndrome" (NBS).

The immunological findings in NBS show the same variability as in AT. Three patients have an hypoimmunoglobulinaemia, one has IgA deficiency and one low normal immunoglobulin levels.

The cellular immunity of the Dutch boy in only slightly impaired, whereas the Czechoslovakian patients have more severe abnormalities.

The cytogenetic and cellbiological findings, thus far, are strikingly similar to AT. However, alphafetoprotein levels are consistently increased in AT, but not in the patients reported here (table 1).

The clinical course of both syndromes appears to be identical: patients die from resporatory tract infections or lymphoreticular malignancies.

Taking all available data together, we believe that NBS is a cell biological/clinical variant belonging to the same disease group as classical AT, but differing from classical AT by neither manifesting ataxia nor telangiectasias and possibly also in other aspects, e.g., reduced head circumference and normal alpha fetoprotein level. Therefore, we would like to reconsider the

definition of the syndromes AT and NBS, taking clinical, immunological, cytogenetic, and cell biological data into account.

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Chapter 6

THE NIJMEGEN SYNDROME
DESCRIPTION OF FOUR NEW FAMILIES AND FURTHER DELINEATION

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submitted for publication.

SUMMARY

In this paper five independent families are described with a chromosome instability disorder which earlier had been called the Nijmegen Breakage Syndrome (NBS). These families -two from the Netherlands and three from Czechoslovakia- had a total of eight patients, five of whom still alive.

The main clinical characteristics were microcephaly, stunted growth, a "bird-like" face, immunological defects involving both the humoral and the cellular system. In four of the five living patients has it been possible to study the chromosomes of cultured lymphocytes. The basic karyotypes in these patients were normal, but in 24 to 35% of the metaphases rearrangements were found, preferentially involving the chromosomes 7 and/or 14 at the sites 7p13, 7q34 and 14q11.2.
The chromosomes of all five living patients were very sensitive to ionizing radiation. In addition, the DNA synthesis in their cultured lymphocytes and fibroblasts was more resistant to X-rays than in cells from normal controls.

The Nijmegen Breakage Syndrome shares a number of important features with ataxia telangiectasia. Both syndromes are characterized by the occurrence of typical rearrangements of the chromosomes 7 and/or 14, cellular and chromosomal hypersensitivity to X-irradiation, radioresistance of the DNA replication and immunodeficiency. However, there are also obvious differences: NBS patients have microcephaly, but neither ataxia nor telangiectasia, and in contrast to the situation in AT the

alpha-fetoprotein level in their serum is normal.

INTRODUCTION

A few years ago some of us reported two sibs with a new, presumably autosomal recessive, chromosome instability syndrome which was provisionally named the Nijmegen Breakage Syndrome (NBS) (Weemaes et al, 1981). The main clinical characteristics were microcephaly, stunted growth, skin abnormalities, immunological defects involving both the cellular and humoral system, and mental retardation. Laboratory studies revealed a high incidence of typical chromosome 7 and/or 14 rearrangements in the cultured lymphocytes (Hustinx et al, 1979; Weemaes et al, 1981), and an abnormally increased rate of cell death and chromosome damage after X-ırradiation (Taalman et al, 1983). In addition, the inhibition of the DNA synthesis after X-irradiation was less pronounced than in normal controls (Taalman et al, 1983). The cytogenetic, cell biological and immunological findings were strongly reminescent of those in ataxia telangiectasia (AT) (Weemaes et al, 1981; Taalman et al, 1983).

Since the first description of the probands with NBS we have been able to study four other families with apparantly the same clinical and cytogenetic entity. Three of the families have been earlier described from Czechoslovakia as having a possible new syndrome with microcephaly, normal intelligence, immunodeficiency and risk for lymphoreticular malignancies (Seemanová et al, 1985), but turned out to fit very well into the Nijmegen Breakage Syndrome (Weemaes et al, 1986).

In this paper we summarize and evaluate the clinical, cytogenetic, cell-biological and immunological data of two families from The Netherlands and the three from Czechoslovakia pertaining to a total of eight patients, five of whom still alive.

PATIENTS

The Dutch families

One of the two Nijmegen families has been presented before (Hustinx et al 1979; Weemaes et al, 1981). The proband (HH) of this family is 17 years now. Since the previous report he has once suffered from a bronchopneumonia. His height is still below P10. His head circumference measures 48.5 cm (<P10). There are still no signs of ataxia nor telangiectasias.

FIGURE 1.
Patient BvH at the age of 2 years

The proband (BvH) of the second Dutch family, a boy, was born in 1984. His parents are not consanguineous. The weight and length at his birth after a pregnancy duration of 35 weeks were 2080 g en 41 cm respectively. His head circumference at birth was 31 cm.

At the age of 5 weeks a hydrocephalus was noticed that was treated by a ventriculo-peritoneal drain. A few months later congenital dysplasia of the hips and a hydronephrosis at the right were found. Since his first months he suffered from various infections: otitis media, repeated pneumonias, gastro-enteritis, oral candida infection, osteomyelitis, and urinary tract infection. He was admitted to our hospital at the age of 21 months (Fig. 1). His height and weight were then 73 cm and 6600 g (both below P10). His head circumference was also below the P10: 36,5 cm.

He had a narrow face, low set ears, retrognathia, a relative large narrow nose and clinodactyly of the right fifth finger.

There was a markedly delayed speech, and partial loss of hearing. There were no signs of ataxia nor telangiectasias.

The Czechoslovakian families

The patients 2 (MD), 7 (JZ), 8 (RZ), and 9 (DL) belonging to the families (2), (4), (5), and (6) in the original paper of Seemanová et al, (1985) were still alive and could be reinvestigated.

The humoral and cellular immunity in one of these patients (DL) turned out to be normal now. Also the cytogenetic and cell biological studies of his cultured lymphocytes gave quite normal

results. We therefore decided to exclude this patient from the present paper.

The remaining three patients belong to three unrelated families. Their clinical data have already been presented in detail (Seemanová et al, 1985) and are summarized in Table 1.

In the five living patients the serum level of alpha-fetoprotein was in the normal range.

	Family 1		Family 2	Family 3		Family 4		Family 5
	MH	HH	BvH	MD	DD	LZ	JZ	RZ
Year of birth	1964	1969	1984	1971	1977	1980	1981	1979
Sex	♂	♂	♂	♀	♂	♀	♂	♀
Birth weight (g)	2500	2500	2080	2340	2740	2450	2400	2000
Birth length (cm)			41	43	48	48	46	46
Gestation duration (weeks)	40	38	35	43	40	40	40	41
Head circumference (cm).								
at birth	↓	↓	31	27	30.5	30	30.5	29
at age (years)	42(6)	49(16)	36,5(2)	42(14)	39(2.5)	35(5/12)	41,5(4)	38(1)
Stunted growth	+	+	+	+	−	−	±	±
Microcephaly	+	+	+	+	+	+	+	+
"Birdlike" face		+	+	+	+	+	+	+
Renal abnormality	+	−	+	−	−	−	−	+
Defect in immunity	+	+	+	+	+	?	+	+
Infections	+	+	+	+	+	+	+	+
Mental subnormality	+	+	±	−	−	−	−	−
Increased level of α-fetoprotein		−	−	−	?	?	−	−
Age at death (years)	6.5				2.25	0.5		
Cause of death	pneumonia				lymphosarcoma	pneumonia		

Table 1

The main clinical data of the patients.

Chromosome studies were performed on peripheral lymphocytes cultured for 72 h or 96 h in RPMI 1640 medium supplemented with 20% fetal calf serum (FCS), or in medium 199 with 5% FCS. The media contained also antibiotics and phytohaemagglutinin. Chromosome preparations were made as usual (Scheres, 1972). Routine chromosome analyses were done on conventional and/or GTG-banded metaphases (Scheres, 1972).

The sensitivity of the chromosomes to X-rays was investigated as follows. The lymphocytes were cultered for about 65 hours in RPMI 1640 medium with 20% FBS, antibiotics and PHA. The cultures were then exposed to X-rays (1 Gy). The irradiation of the blood cultures was performed with a Siemens Stabilipan X-ray machine operating at 300kV, 12 mA and providing a dose rate of 0.5 Gy/min. The incubation of the cultures was then continued for another 6 hours with colcemid present during the last 1.5 hours. The chromosome preparations were made in the usual way and scored for aberrations. The blood cultures from normal controls and AT patients were set up and treated in the same way.

The inhibition of the DNA synthesis after X-irradiation was measured in lymphocytes as well as in fibroblasts using a modification of a method, which has been described earlier (de Wit et al, 1981; Jaspers et al, 1981).
The peripheral lymphocytes were purified using the Ficoll-Isopaque gradient centrifugation method. 1 ml cultures (300,000 cells/ml) were initiated in MEM medium supplemented with 20%

human serum, antibiotics and PHA. After a two day culture period cells were prelabelled with [2.^{14}C]-thymidine (0.025uCi/ml) overnight in order to have an internal standard proportional to the amount of proliferating cells. Then this radioactive medium was removed. The cells were washed once with preheated PBS. Subsequently fresh medium was added. Cultures were exposed to X-rays (10, 20, 30 and 40 Gy) and immediately thereafter 0.4 uCi/ml [^{3}H methyl]-thymidine was added. After a further 4 hours of incubation the cells were harvested with a cell-harvester. The filters were air dried and put in a scintillation vial. Then the scintillation cocktail (Packard) was added and the ratio of ^{3}H to ^{14}C radioactivities determined.

The procedure for the fibroblasts was comparable to the one described for the lymphocytes except that the cells were seeded in 3 cm Petri dishes and the culture medium was Ham's F10 supplemented with 15% FBS and antibiotics. The incubation after ionizing radiation was 4 hours in the presence of 0.8 uCi/ml [^{3}H methyl]-thymidine and the cells were trypsinized before they were collected with a cell harvester.

The immunoglobulin concentrations were determined by an immuno-turbidimetric method using an LKB reaction rate analyzer (van Munster et al, 1977).

The lymphocyte stimulation tests were done according to DuBois (1973). The peripheral blood lymphocytes were obtained from defibrinated venous blood by centrifugation with Ficoll-isopaque. The proliferation of the lymphocytes was studied with

phytohaemagglutinin (PHA) and pokeweed mitogen (PWM). The method of Stjernsward et al (1972) was used for the E rosette tests.

RESULTS

Cytogenetic studies

In only four of the five living patients has it been possible to study the chromosomes of cultured lymphocytes after GTG-banding (Table 2). The basic karyotypes in these patients were normal, but in 17 to 35% of the metaphases chromosome rearrangements were found. The majority of these abnormalities were rearrangements involving the chromosomes 7 and/or 14: inv(7)(p13q34), t(7;14)(p13;q11.2), t(7;14)(q34;q11.2), and t(7;7)(p13; q34)(Tables 2 and 3) (Fig. 2).

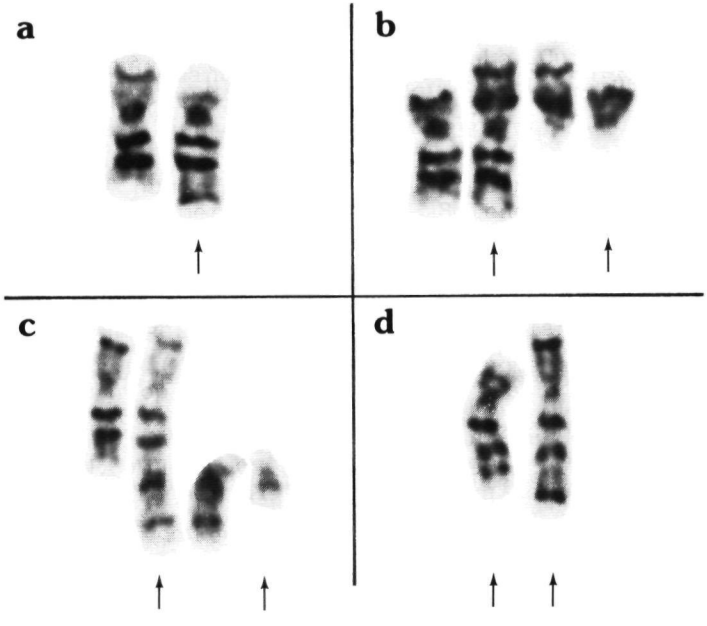

FIGURE 2.
Typical chromosome 7 and 14 rearrangements found in PHA stimulated lymphocytes of the probands.
a. inv(7)(p13;q34), b. t(7;14)(p13;q11), c. t(7;14)(q34;q11), d. t(7;7)(p13;q34).

	HH						BvH	MD	RZ
Age at investigation (years)	9[@]	12	14	15	16	Total	2	14	15
Total number of cells	334	105	15	98	99	651	76	54	42
t(7;14)(q34;q11.2)	9	13	2	8	8	40	1	4	3
t(7;14)(p13;q11.2)	12	3	0	3	5	23	2	4	2
t(7;7)(p13;q34)	4	0	0	0	3	7	2	0	0
inv(7)(p13;q34)	23	16	7	13	4	63	6	4	4
t(14;14)(q11.2;q32)	1	0	0	0	0	1	0	0	0
inv(14)(q11.2;q32)	0	0	0	0	0	0	0	0	0
Complex 7/14 situations *	4	2	0	2	0	8	0	0	0
Other rearrangements *	3	3	0	4	11	21	7	6	3
Percentage of the cells with a 7 and/or 14 rearrangement	15.9	32,4	60	26,5	20,2	21.8	14	22	21
Percentage of cells with a structural abnormality	16.8	35,2	60	30,6	30,3	25.0	23,7	33	28,5

Table 2

The structural chromosomal abnormalities in four NBS patients.
(*For specification see Table 3)

[@] These data are from Hustinx et al, 1979 and Weemaes et al, 1981

a

Initials of the patient	Number of cells	Karyotype
HH	3	46,XY, t(7;7;14)(p13;q34p13;q11)
	1	43,XY, -5,-6,-17, t(7;7;14)(p13;q34p13;q11)
	1	45,XY, -5, t(7;7;14)(q34;p13q34;q11)
	1	46,XY, t(7;14)(p13;q32)
	1	46,XY, inv(7)(p13q34), t(7;14)(q34;q11)
	1	46,XY, inv(7)(p13q34), inv(7)(p13q34)

b

Initials of the patient	Number of cells	Karyotype
HH	1	46,XY, rcp (10;14)(?;q11)
	1	46,XY, rcp (4;14)
	1	46,XY, rcp (14;17)
	2	46,XY, del 14q
	2	46,XY, del 7q
	1	46,XY, t(X,?)
	1	46,XY, i9q
	1	46,XY, i9q, i11q, del 1p
	1	46,XY, i1q
	2	46,XY, del 1q
	1	46,XY, t(1;10)
	1	46,XY, del 2q
	1	46,XY, t(2,?)
	1	46,XY, i13q
	1	46,XY, Tr (5q6q)
	1	46,XY, Tr unspecified
	1	47,XY, + i(9q)
	1	47,XY, + del(1)(q24:)
BvH	1	46,XY, t(X;12)
	1	45,XY, -14, -15, t(4q;15q)
	1	46,XY, rcp (1;17)
	1	46,XY, rcp (12;14)(q+;q11)
	1	46,XY, t(7;?)(q22;?)
	1	46,XY, del 15q
	1	46,XY, -10, marker
MD	2	46,XX, t(2;14)(q+;q-)
	1	46,XX, t(9;14)(q+;q-)
	1	46,XX, t(X;14)(q+;q-)
	1	46,XX, t(13;14)
	1	46,XX, del 2q
RZ	1	46,XX, t(3;3) dic
	1	46,XX, t(12;?)
	1	46,XX, ? (5p)

Table 3

Specification of the complex 7/14 situations (a) and the other rearrangements (b) from Table 2.

Occasionally a translocation was observed between two other chromosomes or between a chromosome 7 or a 14 and another chromosome (Tables 2 and 3). In only one of these patients a cell was seen with 14q32 involved in a rearrangement: tan(14;14)(q11.2;q32).

The sensitivity for X-rays of the chromosomes of cultured lymphocytes was studied by exposing the cells to X-irradiation in the G_2 phase of the cell cycle. Results could be obtained for all five patients (Table 4). The frequency of aberrations was significantly higher in the cells of the patients than in those of the control persons.

The rate of abnormalities found in the patients is similar to the one in cells from patients with ataxia telangiectasia. This

	No. of cells examined	Aberrations per cell	% Cells with aberrations
NBS patients:			
HH	50	1.78	74
BvH	50	1.32	76
MD	100	1.21	64
JZ	55	1.85	69
RZ	100	1.28	67
AT patients:			
MS	76	2.45	88
HC	50	1.42	69
Controls:			
ES	50	0.04	4
CO	50	0.12	12

Table 4

Chromosomal aberrations per cell and percentages of cells with aberrations in G_2 - irradiated cultured lymphocytes from the NBS patients, two AT patients and two normal controls.

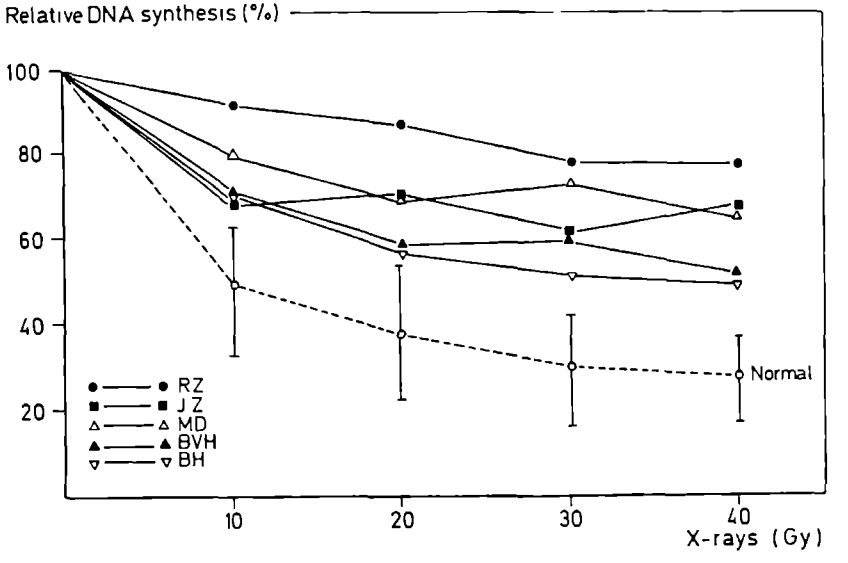

FIGURE 3.
Inhibition of DNA synthesis in cultured lymphocytes after exposure to X-rays. The data of the patients are the mean of triplicate determinations carried out in a single experiment. The control data are the mean of such experiments on 9 control persons.

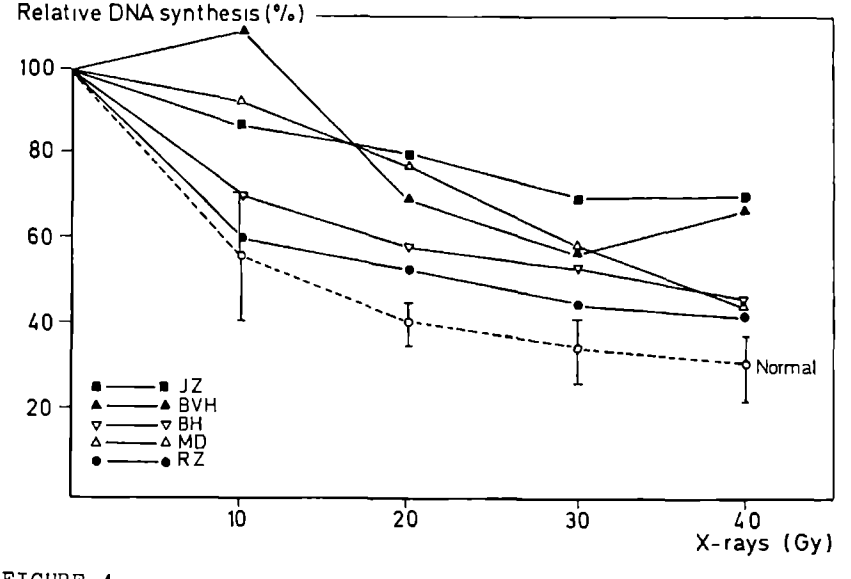

FIGURE 4.
Inhibition of DNA synthesis in cultured fibroblasts after exposure to X-rays. The data of the patients are the mean of triplicate determinations carried out in a single experiment. The control data are the mean of such experiments on 8 control fibroblasts cultures.

is illustrated by the data from two AT patients which are also presented in Table 4.

DNA replication after X-irradiation

For the study of the DNA replication after X-irradiation both PHA stimulated lymphocytes and cultured fibroblasts from the five living patients were used.

In both tissues from all five patients the DNA synthesis after X-irradiation turned out to be less inhibited than in cells from control persons (Figs. 3 and 4). At a dose of 40 Gy the rate of DNA replication in the probands was 43-78% while in normal individuals the DNA synthesis was reduced to 17-38% of the values found in their untreated controls.

Immunological studies

Quantitative serum immunoglobin studies revealed abnormalities in all the patients investigated (Table 5).

Two patients (MD and JZ) had an agammaglobulinaemia, two (BvH and RZ) an IgG subclass deficiency and one an IgA deficiency (HH). Four of the five patients had an IgD and an IgE deficiency, and in the fifth (RZ) the IgD and IgE levels were greatly decreased.

The cellular immunity was deficient in four of the five patients. The percentages of their T cells were decreased and the proliferative responses of the peripheral blood lymphocytes were less than 20% of the controls assayed concurrently. In the

	IgG IU/ml	IgA IU/ml	IgM IU/ml	IgD IU/ml	IgE IU/ml	IgG subclasses (% of normal adult values) IgG_1	IgG_2	IgG_3	IgG_4	% T-cells	Mitogen responses of blood lymphocytes PHA	PWM
HH	109	< 1	66	<1	< 0.5	57	53	104	<10	low to normal	100%	50%
BvH	65	49	50	<1	1	93	<10	77	<10	↓	20%	< 5%
MD	5	< 2	143	<1	< 0.5	< 10	<10	< 10	<10	↓	< 5%	< 5%
JZ	2	9	42	<1	< 0.5	< 10	<10	< 10	<10	↓↓	< 5%	< 5%
RZ	53	28	120	3	8	72	<10	59	<10	↓↓	< 5%	< 5%

Table 5

The immunological findings in the patients.

fifth patient (HH) the percentage of T cells was only slightly decreased. The response of his peripheral lymphocytes to PHA has been always normal but to PWM repeatedly impaired last years.

DISCUSSION

In this paper eight patients from five independent families are described with a chromosome instability syndrome which had been called the Nijmegen Breakage Syndrome (NBS) (Weemaes et al, 1981). Three of the patients are deceased -one from a lymphosarcoma and two from a pneumonia (Table 1).
The five living patients could be studied more thoroughly.

All patients had a low weight and a small length at birth, and their height remained below the 10^{th} percentile. They all were microcephalic and their face had a "bird-like" appearance. In three of the eight patients a renal anomaly, viz. an unilateral hydronephrosis, had been detected.
The psychomotoric development was normal in the Czechoslovakian patients. Though slight mental retardation was present in the Dutch patients it is not necessarily related to the syndrome, as the parents of family H are both of borderline intelligence and the problems in patient BvH might also originate from his hearing loss. Most patients have suffered from repeated infections, predominantly of the respiratory tract.

All five living patients have an immune defect. Two patients have an agammaglobulinemia (MD and JZ), two are IgG2 and IgG4

deficient (BvH and RZ), and one has an IgA deficiency (HH) (Table 5). The cellular immunity is greatly disturbed in four of the five patients (Table 5). Also the deceased patients had shown clinical evidence of impaired immune competence (Table 1); however, their laboratory data are scare. The data indicate that the immunological disturbances in the syndrome can be rather variable.

The chromosome studies in these patients were much hampered by the disturbance in the cellular immunity, which leads to a low rate of blastic transformation and mitoses in the PHA-stimulated lymphocyte cultures. This phenomenon has been mentioned previously by Seemanová et al (1985). Also in the present study we were not able to perform our whole program in one of the patients (JZ) although lymphocyte cultures had been repeated several times. In the PHA-stimulated lymphocyte cultures of the remaining four patients a high frequency of rearrangements was found (Table 2 and 3). Among these two types of translocations between the chromosomes 7 and 14 and two types of rearrangements limited to one or both chromosomes 7 predominated: t(7;14)(p13;q11.2), t(7;14)(q34;q11.2), t(7;7)(p13;q34) and inv(7)(p13q34). (Fig. 2).

We are fairly certain that the exchange points in 7p and 14q in these translocations are p13 and q11.2, respectively. However, defining the exact exchange point on the long arm of chromosome 7 was difficult and in the earlier publications in this field (Hustinx et al, 1979; Weemaes et al, 1981; Taalman et al, 1983;

Weemaes et al, 1986) we specified this exchange point as q32. The present evidence indicates that the exchange point on 7q has to be localized more distally, at least at q34. Until now high resolution banding of the chromosomes from the NBS patients has failed to give better results than the usual GTG banding.

In none of the cells of our patients an inv(14)(q11.2q32) was seen and in only one cell a tandem translocation (14;14)(q11.2;q32). In one patient (HH) several cytogenetic studies have been performed during the last seven years (Table 2). The frequencies of the 7 and 14 rearrangements have not changed distinctly in this period.

The chromosomes of cultured lymphocytes of all our patients turned out to be very sensitive to ionizing radiation (Table 4): after administration of X-rays during the G_2 phase of the cell cycle at least ten times more chromatid breaks occurred in the cells of our patients than in those from normal controls. This frequency is comparable to that found in ataxia telangiectasia (AT) (Taalman et al, 1983; Weemaes et al, 1984; Bender et al, 1985). Hypersensitivity of the chromosomes to X-rays is not restricted to those of cultured lymphocytes as an earlier study of the phenomenon in fibroblasts of HH has shown (Taalman et al, 1983).

The DNA synthesis in the cells (cultured lymphocytes and fibroblasts) from our patients was more resistent to X-rays than that in cells from normal controls (Figs. 3 and 4). Such an effect has been previously described for patient HH (Weemaes et al, 1981; Taalman et al, 1983).

Our NBS patients share a number of important cellular features with ataxia telangiectasia, in particular the occurrence of the characteristic rearrangements involving the chromosomes 7 and 14, the cellular and chromosomal hypersensitivity to X-irradiation, the radioresistance of the DNA replication and the immunodeficiency. Clinically however, they differ clearly from AT patients in having neither ataxia nor telangiectasias, but presenting with microcephaly. Also, in contrast to AT the alpha-fetoprotein level in the serum of the NBS patients is not increased.

In comparing the cytogenetic data from NBS with those from AT the following observations may be pertinent.

The frequency of rearrangements, especially those of the chromosomes 7 and 14 is consistently high in the NBS patients and seems to be more conspicuous than in patients with classical AT (Aurias et al, 1980; Weemaes et al, 1984).

The tan(14;14)(q11.2;q32) is infrequent in our NBS patients and in none of the NBS cases an inv(14)(q11.2;q32) was seen. In NBS the involvement of chromosome 14 in tandem translocations and inversions seems to be less pronounced than in AT (Aurias et al, 1980; Hecht and Kaiser Mc-Caw, 1982; Kohn et al, 1982).

The exchange points most frequently involved in the 7 and 14 rearrangements in NBS are the same as those in AT, but the frequencies of involvement of the individual sites might differ between the two syndromes. These sites correlate in location with genes recognized as sites of gene rearrangements during T-lymphocyte maturation: at 7p13-21 the gene for the T cell receptor gamma chain is localized (Morton et al, 1985), at

7q32-q35 the gene for the T cell receptor beta chain (Morton et al, 1985; Isobe et al, 1985) and at 14pter-q22 the gene for the T cell receptor alpha chain (Jones et al, 1985). At 14q32 the genes for the immunoglobulin heavy chain -genes pertinent to beta cells- are mapped (Wesley McBride et al, 1982).

Obviously, genes with immune functions are implicated in chromosome rearrangements in AT as well as in NBS. We have suggested earlier (Scheres et al, 1986) that the origin of such chromosome 7 and 14 rearrangements might be the consequence of erroneous recombinations involving T- and B-cell genes. This suggestion is shared by several other authors (Fiorilli et al, 1985; Dewald et al, 1986; Hecht et al, 1986).

Localization of the breakpoints that are involved in the other translocations found in AT and NBS (Table 2) might therefore give more insight as to the possible existence and location of other genes important to immunity.

Pertaining to this, we regret that we were not able to specify the exchange points in all the rearrangements found (Tables 2 and 3).

In view of the cellular analogies between NBS and AT complementation studies have been done with fibroblast cell lines from patients with these syndromes (Jaspers et al, 1987). The abnormal radioresistent DNA replication has been used as a parameter for the complementation analysis. None of our NBS patients tested (HH, BvH, MD and JZ) belongs to one of the four known AT complementation groups (Jaspers et al, 1987; Jaspers, personal communication). It was also detected that there exist at least two NBS complementation groups. These are called

arbitrarily V_1 and V_2 (V = Variant of AT; Jaspers et al, 1987). The patients HH, MD and JZ are classified in group V_1 (Jaspers et al, 1987), and patient BvH in group V_2 (Jaspers, personal communication)(Table 6).

	This paper	Wegner et al, 1982	Conley et al, 1986
Microcephaly	+	+	+
Ataxia	-	-	-
Telangiectasias	-	-	-
7 and 14 rearrangements	+	+	+
X ray sensitivity of the chromosomes	+	+	+
Defect in immunity	+	+	+
Elevated α-fetoprotein	-	-	-
Complementation groups (Jaspers et al, 1987)			
V_1	+x)		
V_2	+xx)	+	+

x) HH, MD, JZ

xx) BvH

Table 6

A survey of all NBS patients

Conley et al (1986) and Wegner et al (1982) have described patients who resemble our patients in clinical, immunological, cytogenetic and celbiological aspects. From both these families also complementation studies were performed (Jaspers et al, 1987). They both turned out to belong to complementationgroup V_2 (Jaspers et al, 1987) (Table 6).

	Typical NBS	Maraschio et al, 1986		Say et al, 1986		Iskandar et al, 1980	Ying and Decoteau, 1981	Webster et al, 1982	Byrne et al, 1984			Fiorelli et al, 1985		Curry et al, 1986		Typical AT
		case 1	case 2 *	case 1	case 2	case	case	case	case 1	case 2	case 3	case 1	case 2	case 1	case 2	
Microcephaly	+	+		+		-	-	-	-	-	-	-	-	+	+	-
Ataxia	-	-		-		-	+	-	+	+	+	+	+	+	+	+
Telangiectasias	-	-		-		-	-		-	-	-	-	+	+	+	+
7 and 14 rearrangements	+	+		-			+						+	+	+	+
X ray sensitivity of the chromosomes	+					+		+				-		+	+	+
Defect in immunity	+	+		+		-	+	+	+	+	+	+	-	+	+	+
elevated α-fetoprotein	-	-					+		-	-	-			-	+	+

* lymphocytic lymphoma

Table 7

A survey of patients having affinities to both NBS and AT but who cannot be assigned to one of these syndromes on account of the data presently available.

The patients of Wegner et al (1982) and of Conley et al (1986) can therefore be regarded as having the NBS syndrome, thereby bringing the number of families with the syndrome to seven.

In Table 7 a number of patients are surveyed who express one or more of the most conspicuous features of NBS or AT, but who cannot be assigned to one of these syndromes on account of the data currently available. It is possible that (some of) these patients have another "variant" syndrome, and that the NBS and AT are only part of a spectrum of biologically related human disorders.

Clinically there is also some resemblance between our patients and those with the so-called Dubowitz syndrome. This is an autosomal recessive syndrome characterized by pre- and postnatal growth retardation, microcephaly, a peculiar face, a moderate mental and speech retardation (Dubowitz, 1965; Wilroy et al, 1978), and in some cases also by an immunodeficiency (Majewski et al, 1975; Sauer and Spelger, 1977). Moreover, an increased risk for malignancies has been suggested (Sauer and Spelger, 1977). Only in a few patients chromosome studies were done, and in one of them a slightly elevated rate of spontaneous chromosome breakage was found (Walters and Deposito, 1985). In our opinion the present knowledge warrants extensive study at the spontaneous and inducable chromosome instability in patients suspected of having Dubowitz syndrome.

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Chapter 7

PATIENTS WITH AN INHRITED SYNDROME CHARACTERIZED BY IMMUNODEFICIENCY, MICROCEPHALY AND CHROMOSOMAL INSTABILITY: GENETIC RELATIONSHIP TO ATAXIA TELANGIECTASIA

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SUMMARY

Fibroblast cultures from six unrelated patients having a familial type of immunodeficiency, combined with microcephaly, developmental delay and chromosomal instability, were studied with respect to their response to ionizing radiation. The cells from 5 out of 6 of such patients resembled those from individuals with ataxia telangiectasia (AT) in that they were 2 - 3 times more radiosensitive on the basis of clonogenic cell survival. In addition, they showed an inhibition of DNA replication after exposure to x-rays or bleomycin that was less pronounced than that in normal cells and characteristic of AT fibroblasts. However, the patients are clinically very different from AT, not showing any signs of neurocutaneous symptoms.

Genetic complementation studies in fused cells using the radioresistant DNA synthesis as a marker showed that the patients' cells could complement representatives of all presently known AT complementation groups. Furthermore, they were shown to comprise a genetically heterogeneous group themselves as well.

It is concluded that the patients studied here are similar to AT with respect to cytological parameters. The clinical differences between these patients and patients having AT are a reflection of genetic heterogeneity. The data indicate that the patients suffer from a chromosome instability syndrome that is distinct from AT.

The inherited syndrome ataxia telangiectasia (AT) is clinically characterized by progressive cerebellar ataxia, oculocutaneous telangiectasia, frequent immunodeficiency and chromosomal instability. Patients with the disorder have an enhanced risk of developing neoplasia, particularly in the lymphoreticular system (for reviews, see Sedgwick and Boder, 1972; Gatti et al, 1982). Cultured cells from AT patients are hypersensitive to the cytotoxic and clastogenic action of ionizing radiation and radiomimetic chemical agents, such as bleomycin, adriamycin, neocarzinostatin and hydrogen peroxide (Taylor, 1978; Bridges and Harnden, 1982; Shiloh et al, 1983; Gatti and Swift, 1985). An almost consistent biochemical abnormality found in AT cells is a relative resistance of DNA synthesis to the agents mentioned (Painter, 1981; Cramer and Painter, 1981; Jaspers et al, 1982; Shiloh et al, 1982). Complementation analysis using the abnormal pattern of DNA replication as a marker has revealed that AT is genetically heterogeneous (Jaspers and Bootsma, 1982; Murnane and Painter, 1982). Sofar, five different complementation groups have been identified (Jaspers et al, 1985; Jaspers and Baan, unpublished data).

In the recent years a number of patients have been described suffering from an inherited syndrome resembling AT in some respects, but missing one or some of the cardinal clinical symptoms of AT (Daneshbod-Skibba et al, 1980; Weemaes et al, 1981; Sperling, 1983; Byrne et al, 1984; Helmerhorst et al, 1984;

Seemanová et al, 1985; Conley et al, 1986). Subject of this report are patients in this category showing immunological impairment and chromosomal instability resembling that in AT, but no clinical signs of neurocutaneous involvement. In addition, these patients have microcephaly and developmental delay as a common feature. This type of disorder was first described by Weemaes et al (1981) who tentatively called it the "Nijmegen breakage syndrome" (NBS).

Here we report the results of studies on cultured fibroblasts of another five of such patients, originating from various countries, whose clinical characteristics, as published by others earlier (Weemaes et al, 1981,1986; Sperling, 1983; Seemanová et al, 1985; Conley et al, 1986), resemble those of the original NBS patient in many respects, although there are variable clinical differences as well. The response to X-rays was investigated and the cell strains found hypersensitive were subjected to complementation analysis. The data suggest, that the patients studied here resemble AT patients with respect to the cellular radiosensitivity, but comprise a separate genetic category. Moreover, the patients could be assigned to two separate complementation groups.

MATERIALS AND METHODS

Cell strains and culture conditions.

A list of fibroblast strains used is given in Table 1. The complementation group assignments of the AT cell strains were either reported earlier (Jaspers and Bootsma, 1982; Jaspers et

TABLE 1. LIST OF CELL STRAINS USED

Patient cell strains:
79RD27, "Nijmegen breakage syndrome" (NBS), Weemaes et al, 1981
2239, Sperling, 1983
1129W, patient 7 in: Seemanová et al, 1985
1217G, patient 2 in: Seemanová et al, 1985
1129X, patient 9 in: Seemanová et al, 1985
GM7166, Conley et al, 1986

Ataxia Telangiectasia:
Group A: AT3BI, AT5LA
Group B: AT2BE
Group C: AT4BI, AT3LA
Group D: AT5BI, AT6BI
Group E: AT8BI

Normal cell strains:
C7RO, 77RD224, 77RD218

TABLE 2: PATIENT CHARACTERISTICS: LITERATURE DATA a)

	2239	79RD27	1129W	1217G	1129X	GM7166
Patients' initials	MH	HH	JZ	MD	DL	DM
Nationality b)	G	NL	CZ	CZ	CZ	USA
Sex	F	M	M	F	M	F
Telangiectasia	-	-	-	-	-	-
Cerebellar ataxia	-	-	-	-	-	-
Mental retardation	-	-/+	-	-	-	-/+
Microcephaly	+	+	+	+	+	+
Growth retardation	+	+	+	+	+	+
Peculiar face	+	+	+	+	+	+
Infections	+	+	+	+	+	+
Immune defect	+	+	+	+	?	+
Malignancies	-	-	-	-c)	-	-
Chromosomal instab.	+	+	+	+	-	-
Elevated AFP	-	-	-	-	-	-

a) and R.D.Wegner (univ.Berlin), pers.comm.
b) G=Germany, NL=The Netherlands, CZ=Chechoslovakia
c) Affected sib has developed fatal lymphoreticular neoplasia

al, 1985) or based on our unpublished observations. The clinical characteristics of the patients under study are summarized in Table 2. Some of the patients' cell strains were kindly supplied to us by Drs. E.Seemanová, E.Passarge and R.D.Wegner. GM7166 was obtained from Human Genetic Repository in Camden.

Cells were routinely cultured in Ham's F10 medium supplemented with 15 % fetal bovine serum and antibiotics. All fibroblast cultures were free of contamination with mycoplasma, as checked with Hoechst 33258 fluorescence microscopy.

Inhibition of DNA synthesis.

The method for determining the rate of DNA replication was described earlier (Jaspers et al, 1982). In short, cells were prelabeled with 14C-thymidine and exposed to graded doses of X-rays or bleomycin. Then they were cultured for 4 hrs in presence of tritiated thymidine and harvested. The ratio of 3H to 14C radioactivities was taken as a measure of the rate of DNA synthesis and expressed as a percentage of the values found in untreated controls.

X-rays were delivered from a Philips machine operating at 300 kV, giving a dose rate of 1.75 Gy/min. Bleomycin stock solutions were stored at -20 degr in aliquots to be used only once. Incubation with the drug was for 1 hr.

Survival experiments.

Cellular survival was measured using a thin feeder layer technique described earlier (Jaspers et al, 1982). Exponentially

growing cultures were exposed to x-rays, trypsinized and seeded onto feeder fibroblasts. Colonies of at least 50 cells visible after 11 to 18 days were stained with Coomassie blue and counted. Cloning efficiencies in unirradiated cells varied from 5 to 33 percent.

Complementation analysis.

Complementation experiments were carried out according to a protocol described in a previous report (Jaspers and Bootsma, 1982). In short, two cell strains were preloaded with polystyrene microspheres of different sizes for 3 days, trypsinized, mixed and fused using inactivated Sendai virus. One day later they were exposed to X-rays and cultured with 3H-thymidine for 2 hrs, subsequently fixed and processed for autoradiography. Silver grains were counted above at least 40 nuclei in S-phase in the heterodikaryons and in the two types of homodikaryons, identified on the basis of their contents of plastic beads. The expected average number of grains in the heterokaryons was approximated using the formulae: $exp.Mean=(M1+M2)/2$ and $exp.SEM=SQRT[(S1^2+S2^2)/2+(M1-M2)^2/(4N-4)]$, where M and S are the values obtained in the two types of homodikaryons and N is the number of nuclei counted in the heterodikaryons.

RESULTS

Inhibition of DNA Synthesis

In previous experiments (Taalman et al, 1983) it had been shown that cells from the patient with the 'Nijmegen breakage

syndrome' (NBS) (Weemaes et al, 1981) were hypersensitive to X-rays in terms of cellular survival. This cell strain (79RD27) also showed a relatively radioresistant pattern of DNA replication, typical of AT cell strains. In order to test whether the fibroblasts from the present series of patients,

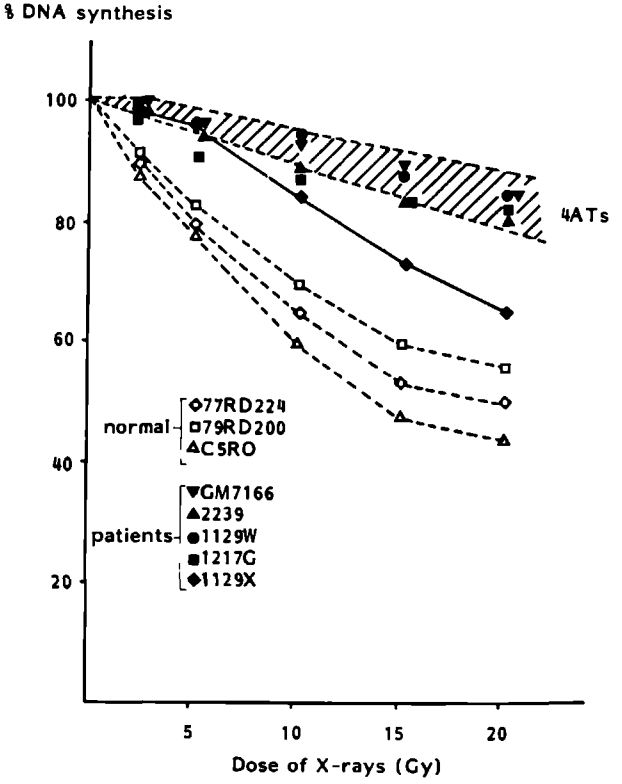

FIGURE 1.
Inhibition of DNA synthesis by X-rays.
Cells prelabelled with 14C-thymidine were exposed to graded doses of X-rays and cultured in the presence of 3H-thymidine for 4 hrs. The rate of DNA synthesis was estimated by the ratio of 3H to 14C radioactivity and expressed as a percentage of untreated cells. Open symbols: cells from normal individuals. Closed symbols:cells from patients. The shaded area represents the responses of four different AT cell strains (AT5BI, AT6BI, AT3LA and AT4BI). The patient data are a mean of at least two independent experiments.

having clinical symptoms similar to the NBS patient are also sensitive, the inhibition of DNA synthesis by X-rays was tested. Fig.1 shows, that the cell strains 1217G, 1129W, 2239 and GM7166 all exhibited an extent of DNA synthesis inhibition that was within the range observed for 4 different AT cell strains tested in the same experiment. 1129X cells showed an inhibition that was intermediate between AT and normal cells.

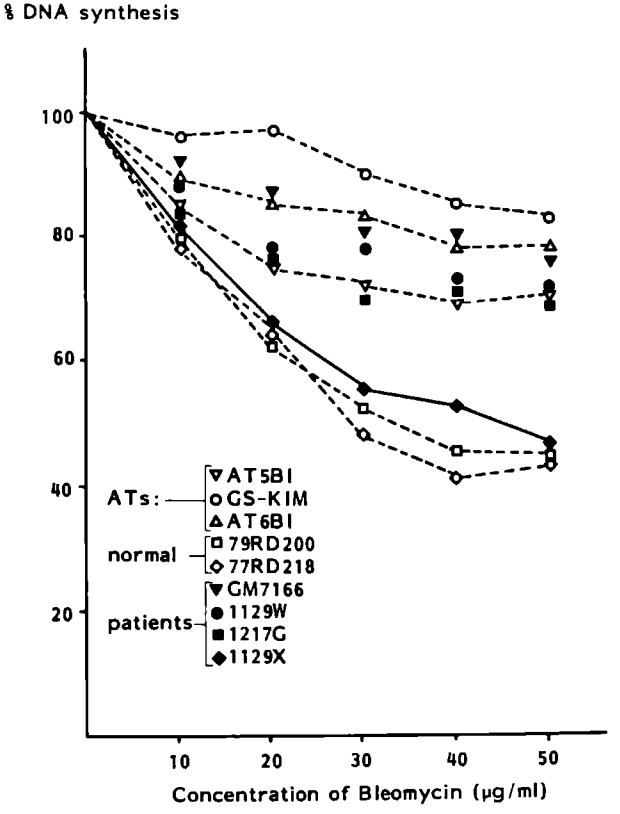

FIGURE 2.
Inhibition of DNA synthesis by bleomycin.
Experimental conditions were as with Fig.1. Treatment with bleomycin lasted 60 min. Open symbols: cells from patients with AT or normal individuals. Closed symbols: cells from patients. Cell strain GS-KIM is identical to AT3LA.

The inhibition of DNA synthesis by bleomycin was also less pronounced in the patients' cells than in cells from normal individuals, and comparable to that in AT fibroblasts (see Fig.2). As an exception, the cell strain 1129X could not be discriminated from the two normal cell strains after treatment with this agent.

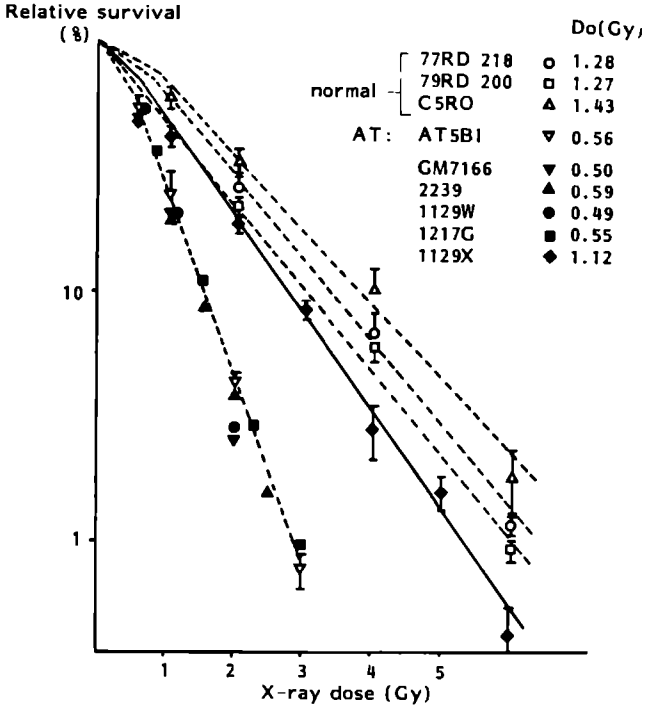

FIGURE 3.
Clonogenic cell survival after exposure to X-rays.
Open symbols: cells from AT or normal individuals. Closed symbols: cells from patients. All patient data represent the mean of at least two independent triplicate experiments. Error bars are given wherever space allows and represent the standard error of the mean. The DO values were calculated from linear part of the semilogaritmic plots using least-squares regression.

Cellular survival

The colony-forming abilities of the patients' cells were measured and compared to AT and normal cells. The results in Fig 3 indicate, that the strains GM7166, 2239, 1129W and 1217G were as sensitive to X-rays as the AT cell strain AT5BI and 2 - 3 times more sensitive than three normal control strains. The DO values were within the range reported for AT by us (Taalman et al, 1983) and others (Bridges and Harnden, 1982) earlier. Again, the 1129X cells showed an exceptional behaviour in that their radiosensitivity was only slightly different from the normal controls.

Complementation analysis

Since most of the patients' cell strains are now found to exhibit a type of radioresistant DNA synthesis, comparable to that observed in AT, there is the possibility to use the rate of DNA replication as a marker in complementation analysis, as has been done in the genetic analysis of AT (Jaspers and Bootsma, 1982; Murnane and Painter, 1982; Jaspers et al, 1985). To this end the rate of 3H-thymidine incorporation in single binucleated cells was measured using autoradiography and the different types of fused cells were discriminated on the basis of their cytoplasmic beads. Fig.4 shows an example of a heterodikaryon.

Table 3 presents the results of a series of fusions with the cell strain 79RD27. In some cases the number of silver grains above the irradiated heterodikaryons was not different from the expected value, defined as the average of the values in

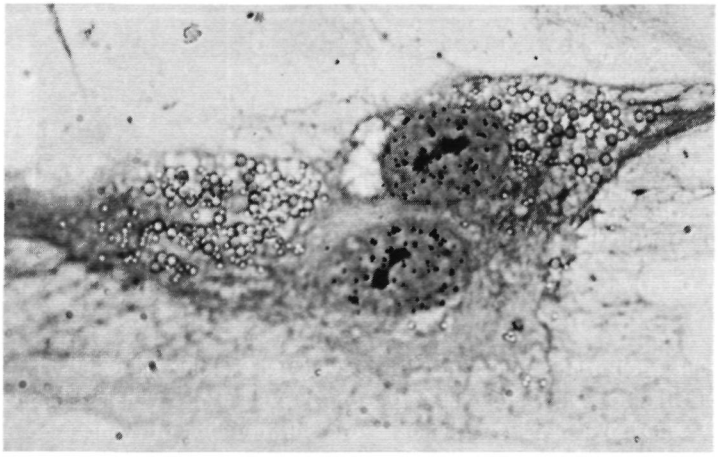

FIGURE 4.
Heterodikaryon obtained after fusion of the cell strains 1217G (small plastic beads) with AT4BI (large beads). Both nuclei are in S-phase as shown by the presence of autoradiographic grains.

the two types of homokaryons. This result, indicating a failure to complement, was obtained after fusion with 1129W. With other fusion partners the observed grain numbers in the heterokaryons were significantly lower than expected, indicating restoration of a normal (strong) type of DNA synthesis inhibition. In this way complementation was observed after fusion with AT cell strains from groups A, C, D and E.The results of similar fusions carried out with the other patients'

TABLE 3. COMPLEMENTATION OF 79RD27

Partner Cell Strain	X-ray Dose (Gy)	AVERAGE GRAIN NUMBER PER NUCLEUS (Mean/SEM) HOMODIKARYONS 79RD27	HOMODIKARYONS partner	HETERODIKARYONS observed	HETERODIKARYONS expected	Signif. (t-test)
AT3BI(A)	40	38.7/2.6	80.9/4.3	43.6/3.8	58.9/3.6	p<0.05
AT4BI(C)	40	33.5/2.1	28.3/1.6	22.8/1.8	30.9/1.9	p<0.05
AT6BI(D)	40	38.6/2.4	29.2/3.3	20.1/2.0	33.9/2.9	p<0.005
AT8BI(E)	40	34.6/2.1	41.2/2.1	26.9/2.0	37.9/2.1	p<0.005
1129W	40	41.8/3.7	47.9/4.8	42.7/3.5	44.8/4.2	Not Sign
GM7166	40	48.8/2.7	49.3/2.7	38.7/2.4	49.0/2.7	p<0.01
2239	40	46.5/3.0	37.0/2.1	31.5/1.9	41.7/2.7	p<0.01

cells are summarized in Table 4. Complementation was seen after fusion with all the AT cell strains tested. These AT cells were from representatives of the complementation groups A, B, C, D and E. Cross-testing the patients' cells revealed that complementation occurred after fusing 79RD27 with GM7166 and with 2239 (see also Table 3). 1129W fails to complement 79RD27, indicating that these two cell strains belong to the

TABLE 4. COMPLEMENTATION ANALYSIS a)

	NBS	1217G	1129W	2239	GM7166
AT-A	+	+	+	+	+
AT-B			+	+	
AT-C	+	+	+	+	+
AT-D	+	+	+	+	+
AT-E	+			+	+
GM7166	+			-	
2239	+		+		-
1129W	-	-		+	

a) -, no complementation; +, complementation to a significance level of $p \leq 0.05$; blank, not tested.

same complementation group, different from the one with GM7166 and 2239, that do not complement each other. The two Chechoslovakian cell strains 1129W and 1217G also do not complement. Altogether the results indicate that the patients can be assigned to two different complementation groups, designated as V1 and V2 (see Table 5), different from the five AT groups.

TABLE 5. COMPLEMENTATION GROUPS.

Group V1	NBS, 1129W, 1217G
Group V2	2239, GM7166

DISCUSSION

The patients studied here were independently identified by different clinicians on the basis of a familial pattern of immunodeficiency, combined with developmental delay and microcephaly and some other variable features. In some of these patients also a chromosomal instability was observed and the presence of lymphocytic clones with chromosomal translocations involving the chromosomes 7 and 14 was noted as well. In this respect a parallel was drawn to the well-known genetic disorder ataxia telangiectasia. To further investigate the relationship to AT we have studied the response to ionizing radiation of cultured fibroblasts from these patients. In an earlier report we described radiosensitivity in terms of cellular survival, induction of chromosomal breakage and

DNA synthesis inhibition in the cell strain 79RD27 from the NBS patient (Taalman et al, 1983). The present data show that cellular radiosensitivity is a characteristic of the other patients studied here as well. Both clonogenic cell survival and the inhibition of DNA synthesis were abnormal and in the range observed in cells from patients having AT. The rate of DNA synthesis was relatively resistant to bleomycin, as has been reported for AT as well (Cramer and Painter, 1981; Jaspers et al, 1982; Shiloh et al, 1983). In one of the cell strains the radiosensitivity was much less pronounced and intermediate between AT and normal controls. The cellular survival was at the borderline for AT patients, and the DNA synthesis inhibition was intermediate between AT and normal cells after X-rays and within the normal range after exposure to bleomycin. Further studies by one of us (Weemaes et al, 1986 ; RDFMT,to be published elsewhere) have shown that in this patient there were neither consistent immunological disturbances (except a lowered IgD) nor signs of chromosomal instability. Furthermore, the inherited nature of the disease was not yet established (Seemanová et al, 1985). It seems probable that some other disease condition is present here.

The present data indicate that on the cytological level these patients cannot be easily distinguished from AT sofar. However, clinically they appear definitely distinct in that they do not show the neurocutaneous hallmarks of AT. In addition, microcephaly and developmental delay are symptoms not regarded to be part of the AT syndrome (Sedgwick and Boder, 1972). There is the possibility that the patients are a reflection of extensive

clinical heterogeneity, that may exist in AT. Alternatively, they may suffer from one or more new and different genetic syndromes In order to distinguish between these two possibilities we have undertaken a genetic complementation study. This approach is feasible since these and AT patients share the feature of radioresistant DNA synthesis, that can be used as a marker in such experiments, as reported earlier (Jaspers et al, 1985). Our data show that the fibroblasts from all the patients can complement representatives from all presently known complementation groups in AT. This result supports the idea that the presently described disease conditions are genetically distinct from AT. The one cell strain 1129X could not be studied in this respect because of its weak radiosensitivity.

AT has been shown genetically heterogeneous. So far five different complementation groups, named A to E, have been identified in experiments using various aspects of the radiosensitivity as a marker (Jaspers et al, 1985, and our unpublished data). Crossfusions with different cell strains from the patients studied here show again genetic heterogeneity. Two separate complementation groups could be discerned, tentatively called V1 and V2 (V for variant). Group V1 harbours patients originating from the Netherlands and Czechoslovakia.Sibs of the Czechoslovakian patients have died from lymphoreticular neoplasia at a young age (15). It is also to be expected that the NBS patient who belongs to the same group has a high risk of developing malignancies as well. In addition, the Czechoslovakian patients exhibited a chromosomal instability that closely

resembled that in the patient with the NBS (Weemaes et al, 1986). In group V2 there is a patient identified in Germany and an American patient, that was reported to be of eastern-European ancestry as well (Conley et al, 1986). It will be interesting to know whether patients with these clinical characteristics can also be found in other geographical locations or ethnic groups. In this respect, patients described in some other recent reports (Maraschio et al, 1986; Teebi et al, 1987), resembling those reported here , would be worth a further study.

In conclusion, the present data support the idea that patients characterized by a familial type of immunodeficiency, combined with early onset microcephaly and developmental delay, as first described in the NBS patient, suffer from a syndrome that is genetically distinct from AT. By its nature this conclusion remains preliminary. More extensive complementation studies on patients with AT or AT-like variant syndromes may produce a different picture: e.g. a classical AT patient could perhaps in the future be assigned to one of the groups V1 or V2. Whatever the final picture may be, our data further point to a high complexity in the response of cells to ionizing radiation and radiomimetic chemical agents. Basic defects in this response can be reflected by a wide spectrum of clinical abnormalities.

ACKNOWLEDGEMENTS

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Chapter 8

CHROMOSOME STUDIES IN IGA DEFICIENT PATIENTS

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Chromosome studies in IgA-deficient patients

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Chromosome analysis was performed in 17 children with IgA-deficiency. In two patients a constitutional structural chromosome abnormality was found. A ringchromosome 22 was seen in one, while in the other a mosaicsm of ringchromosome 18/18p+ was observed. Both patients were mentally retarded and showed distinct congenital defects. From ten asymptomatic patients, spontaneous as well as X-ray induced chromosome instability was investigated. There was no increased spontaneous instability but after irradiation, enhanced chromosome breakage was found in two patient. The elevated frequency of the induced chromosome aberrations in these IgA-deficient individuals however is moderate when it is compared to the increased chromosome damage in patients with ataxia telangiectasia after ionizing radiation.

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Key words: chromosome instability; constitutional chromosome abnormalities; selective IgA-deficiency; X-ray sensitivity at the chromosome level.

IgA-deficiency is the most frequent primary immunodeficiency, and in the normal population serum IgA was shown to be low or undetectable in 1 in 3000 to 1 in 300 (Fine et al. 1973). Both sporadic and familial cases showing variable patterns of inheritance and with or without clinical symptoms have been reported (Grundbacher 1972). Absence of serum IgA has also been described in patients with certain constitutional chromosome abnormalities such as deletions of the long arm of chromosome 18, 21 or 22 (Feingold 1969, Lewkonia 1980, Stricker & Linker 1982, Kadotani & Watanabe 1984, Miranda et al. 1982). In addition, it is well known that IgA-deficiency has a very high prevalence in patients with the chromosomal breakage disorder ataxia telangiectasia (AT) (McFarlin et al. 1972, Boder 1975). Recently, cytogenetic studies on a large group of individuals with IgA-deficiency showed normal chromosome patterns in both symptomatic and asymptomatic cases (Herrmann et al. 1982). However, in this study no attention has been given to the possibility of chromosome breakage phenomena.

In the present study, 17 children with primary IgA-deficiency were karyotyped. In ten of them we also studied spontaneous chromosome breakage and X-ray-induced chromosome damage.

Material and Methods

Patient Group

The 17 patients who were involved in this study were randomly selected from a group of 43 IgA-deficient individuals. All these patients were regularly seen at the Depart-

ment of Pediatrics The clinical findings of these 17 patients are summarized in Table 1

Two patients were mentally retarded and showed distinct congenital defects (P M and E B) Another patient (E J) with congenital abnormalities was IgA-deficient at the time of investigation but at the age of 5 serum IgA rose to 7 Ie/ml The IgA-deficiency of three patients (C E, W Le

Table 1

Clinical state of the patients observed

Patients	Age of IgA-def detection	Indication for immune study	Main physical findings	Clinical symptoms last year	Remarks
C E	7	group study*	none	urinary tract infection	
W Le	9	group study*	none	hay-fever	
C S	9	group study	none	hay fever	
E J	2	recurrent sinopul monary infections	ventriculo-septum defect microcephaly, mental retardation	sinopulmonary infections	not IgA-def at this moment
V P	10	arthralgia general complaints	none	none	
P M	9	recurrent pneumonia caused by an initially unnoticed aspirated corpus alienum	microcephaly, large low-set ears, epicanthal fold, syndactyly of toes, unstable gait	none	
E B	4	sinopulmonary infections	short stature, muscular hypotonia, characteristic facies with prominence of the chin, hypertelorism poor dental occlusion	died of chronic aggressive hepatitis at the age of 7	
D J	8	sinopulmonary infections, fever of unknown origin	none	sinusitis	
D G	2	recurrent cervical abscess, rectal bleeding	none	chronic otitis media	
M J	7	rectal bleeding	none	none	
T J	7	family investigation	none	none	brother of M J
P G	4	fever of unknown origin, otitis	none	none	
Tj J	9	sinusitis	none	bronchitis	
N T	<1	mother with IgA-def and anti IgA antibodies	none	recurrent otitis	
S B	11	bronchitis	none	none	
W L	6	mother with IgA-def and anti IgA antibodies	chronic bronchitis	chronic bronchitis	
A L	4	family investigation	none		sister of W L

*IgA-deficiency of these patients has been detected during a study on the growth and development of normal Dutch children A total of 486 children were investigated 4 of them were found to be IgA-deficient (Weemaes et al 1979)

Table 2

Levels of immunoglobulins, clinical data and karyotype in 17 patients with selective IgA-deficiency

Patients	Age	IgA le/ml	IgG le/ml	IgM le/ml	Karyotype
C E	13	<2	238	104	46,XX
W Le	16	<2	170	101	46,XY
C S	16	<2	173	249	46,XX
E J	4	<2	102	110	46,XX
V P.	14	<2	261	119	46,XX
P M	11	<2	173	163	46,XY,r22
E B.	4	<2	198	160	46,XX,r18,46,XX,18p+
D J	6	<2	182	92	46 XY
D G.	7	<2	177	156	46,XX
Ta J	10	<2	172	133	46,XY
M J	12	<2	216	115	46,XY
P G.	10	<2	179	210	46,XY
Tj J	10	<2	143	64	46,XY
N T	5	<2	162	106	46,XY
S B	13	<2	160	96	46,XY
W L	8	<2	176	187	46,XX
A L.	5	<2	117	138	46,XX

and C. S.) was noted on a longitudinal interdisciplinary study on the growth and development of normal Dutch children (Weemaes et al 1979) Familial IgA-deficiency occurred in five patients (W. L., A. L., Ta. J., M. J. and N. T.).

Normal healthy volunteers, students and employees from the Departments of Human Genetics and/or Pediatrics, were taken as control persons.

Immunoglobulin Studies

Immunoglobulin concentrations were determined by an immunoturbidimetric method using an LKB reaction rate analyzer (van Munster et al. 1977).

Cytogenetic Studies

Lymphocytes were cultured in RPMI 1640, 20% fetal bovine serum (FBS) or in TC 199, 5% FBS supplemented with antibiotics and PHA. Chromosome preparations were made and stained with a trypsin-Giemsa banding technique according to Scheres (1976). At least 10 cells were carefully analyzed.

In order to study spontaneous chromosome instability, the lymphocytes were cultured in TC 199, 5% FBS. Preparations were made in the standard manner, and 100 unbanded Giemsa stained cells were scored for aberrations Blood cultures from the normal healthy volunteers were set up and treated in the same way.

The sensitivity of chromosomes to X-rays was investigated as follows. Lymphocytes were cultured for about 65 h in RPMI 1640 medium holding 20% FBS, antibiotics and PHA. Cultures were then exposed to X-rays (1Gy) (the X-rays were generated by a Siemens Stabilipan X-ray machine operating at 12 mA and providing a dose rate of 0.5 Gy/min), and incubation was continued for a further 6 h. Chromosome preparations were made in the usual way, and 100 metaphases (irradiated in the S/G2 phase of the cell cycle) were scored for each individual. Chromosome aberrations (chromosome breaks, acentric fragments, rings and dicentrics) as well as chromatid aberrations (chromatid breaks, triradials and quadriradials) were counted as one aberration. Chromosome and chromatid gaps, however, were not considered as true aberrations and therefore not included in the

a b

Fig. 1. Chromosomes no 22 from patient P M a) normal chromosome 22, b) ring chromosome 22

a b a c

Fig. 2. Chromosomes no 18 from patient E B , a) normal chromosome 18, b) ring chromosome 18, c) 18p+

total number of aberrations The percentage of aberrant cells in the IgA-deficient individuals was compared to the percentage of aberrant cells in the normal controls (Chi-square).

Results

The results of the immunoglobulin studies in the seventeen patients are listed in Table 2. All 17 patients showed an IgA level below 2 Ie/ml but no other Ig-deficiencies were noted.

The chromosome studies showed a normal karyotype in fifteen patients (46,XX or 46,XY). In the two patients with congenital anomalies, a constitutional structural chromosome abnormality was found in all cells (Table 2). A ring chromosome 22 (Fig. 1)

Table 3

Spontaneous chromosomal instability in cultured peripheral blood lymphocytes (culture medium TC 199 supplemented with 5% FBS, antibiotics and PHA) from 10 IgA-deficient patients and 10 normal controls

	No of cells scored	Aberrations per cell	Cells with aberrations (%)	Cells with more than one aberration (%)
Patients				
D J	100	0 01	1 0	0 0
D G	100	0 21	19 0*	2 0
Ta J	104	0 13	12 0	1 0
M J	100	0 07	7 0	0 0
P G	101	0 33	21 0*	6 0
N T	103	0 17	15 0	1 0
Tj J	103	0 08	6 0	1 0
S B	100	0 11	8 0	2 0
W L	100	0 14	12 0	1 0
A L	100	0 07	7 0	0 0
Range		0 01–0 33	1 0–21 0	0 0–6 0
Mean		0 13	10 8	1 4
Controls				
M K	105	0 04	4 0	0 0
J C	100	0 12	11 0	1 0
J C	100	0 08	8 0	0 0
M W	100	0 17	17 0	0 0
T S.	100	0 04	4 0	0 0
F K	100	0.19	16 0	3 0
B W.	100	0 22	22 0	0 0
H S	100	0 14	12 0	2 0
J S	102	0 11	11 0	0 0
R H	100	0 08	8 0	0 0
Range		0 04–0 22	4 0–22 0	0 0–3 0
Mean		0 12	11 3	0 6

*Significantly greater than controls (pooled results) $p < 0\ 01$

Table 4

Chromosomal aberrations per cell and percentages of cells with aberrations in G2/S-irradiated peripheral lymphocytes from ten IgA-deficient patients and six normal controls (cells were cultured in RPMI 1640 medium with 20% FBS, antibiotics and PHA)

	No. of cells scored	Aberrations per cell	Cells with aberrations (%)	Cells with more than one aberration (%)
Patients				
D.J	119	0 47	31.0*	8.0
D G.	102	0.43	34 0*	8.0
Ta J.	117	0 26	20 0	3.0
M J.	104	0 25	18 0	5 0
P G.	101	0 26	22 0	3 0
N T.	108	0 26	22 0	3.0
Tj.J.	100	0 26	16 0	2.0
S B.	100	0.24	21.0	2 0
W L.	100	0 10	10 0	0.0
A.L.	100	0 26	22.0	3.0
Range		0.10–0.47	10.0–34 0	0 0–8.0
Mean		0 28	21.6	3.7
Controls				
E.K.	100	0.27	16.0	5 0
M.K.	107	0 24	21.0	3 0
J C.	100	0 19	19.0	0.0
J C.	100	0 24	23 0	1.0
M W.	100	0 16	16 0	0 0
T S.	100	0 27	20.0	2 0
Range		0.16–0 27	16 0–23 0	0.0–5 0
Mean		0 23	19 2	1 8

*Significantly greater than normal controls (pooled results) $p < 0.01$.

was seen in the cells of P.M., and a ring chromosome 18 was found in 90% of the lymphocytes from E.B., while in the remaining 10% of her cells an 18p+ was observed (Fig. 2). The quality of the chromosome preparations did not allow exact determination of the deleted segments in the ring chromosomes. Also, the composition of the 18p+ chromosome could not be indicated.

In ten patients, spontaneous as well as X-ray-induced chromosome instability was investigated. The mean percentage of cells with spontaneous aberrations was 10.8% (range 1.0–21.0) in the IgA-deficient patients and 11.3% (range 4.0–22.0) in the controls (Table 3). There was no statistically significant increase in cells with aberrations when the data from the patients were compared to those from the controls.

After X-irradiation in S/G2 of the cell cycle, an increase in chromosome damage was observed in two patients (D.G. and D.J.) (Table 4). There was a significant rise at the $p < 0.01$ level in these individual cases when their percentage of aberrant cells was compared to the mean percentage of cells with aberrations in controls. In lymphocytes from IgA-deficient patients, the aberrations per cell score ranged from 0.10 to 0.47 with a mean percentage of cells with aberrations of 21.6%, and in controls from 0.16 to 0.27 with a mean percentage of cells with aberrations of 19.2%.

Discussion

Fifteen of the IgA-deficient patients showed a basically normal karyotype. Chromosome abnormalities, however, were found in two patients with distinct congenital malformations. A mosaicism of ring chromosome

18 and 18p+ was present in cultured peripheral lymphocytes of E B This patient had many of the clinical features of the 18q deletion syndrome and striking resemblances to the patients described by Lewkonia et al (1980), Stricker & Linker (1982), and Kadotani & Watanabe (1984) In our case, as well as in the patients of Lewkonia et al (1980) and of Stricker & Linker (1982), a selective IgA-deficiency was found Association of selective IgA-deficiency and chromosome 18 abnormalities seems to be frequently seen, albeit inconstantly The extent of the deficiency in the 18q deletion syndrome may perhaps be the consequence of the variation in size of the deleted segment(s) or caused by factors like defective synthetic regulatory mechanisms

The karyotype of the second patient (P M) with congenital malformations showed a ring chromosome 22 A low level or an apparent absence of IgA has been reported in patients with ring chromosome 22 (Dallapiccola et al 1977) and monosomy 22 (Miranda et al 1982) respectively The coincidence of chromosome 22 abnormalities and immunological disturbances is of particular interest because chromosome 22 bears the lambda globulin genes (Erickson 1981) Since the homologous chromosome 22 in the cases of Dallapiccola et al (1977) and Miranda et al (1982) as well as in our patient P M is still intact and presumably functional, the relation between IgA-deficiency and chromosome 22 abnormalities may be complex or labile

A constitutional chromosome abnormality was found only in these two IgA-deficient patients, both having concomitant congenital malformations and mental retardation In view of this, we would strongly recommend consideration of chromosome analysis in mentally retarded patients with IgA-deficiency

The investigation of spontaneous chromosome instability did not reveal a clear and consistent relation between IgA-deficiency and increased chromosome breakage It is apparent that both in the controls as well as in the patient group a wide intra-individual variety exists in the number of cells with aberrations The range and the mean number of cells with aberrations in the controls are almost identical to those in the patients In fact, the mean number of cells with aberrations in controls is even higher than in patients with IgA-deficiency

Two IgA-deficient individuals showed a high incidence of chromosome breakage after X-irradiation The percentage of cells with aberrations in X-irradiated lymphocyte-cultures from these IgA-deficient individuals is about 1 5 times increased over the background level A hypersensitivity to X-rays has been reported for patients with AT as well as for the AT-like Nijmegen breakage syndrome (NBS) (Taylor et al 1975, Taalman et al 1983) In both disorders, immunodeficiency – in most cases IgA-deficiency – is one of the characteristic clinical symptoms (McFarlin et al 1972, Weemaes et al 1981) X-irradiation of cultured cells from patients with these chromosome instability disorders usually induces 8 to 10 times more chromosome damage than in cultured cells from normal controls (Taylor et al 1975, Taalman et al 1983, Nagasawa et al 1985, Parshad et al 1985) This means that the frequency of X-ray-induced chromosome aberrations in AT and NBS is quite different from that in our IgA-deficient individuals Furthermore, there is no statistically significant difference in any IgA-deficient patients when their values are compared to highest control value Therefore, the elevated frequency of chromosome aberrations in the lymphocytes of D G and D J is not considered as a real X-ray hypersensitivity

In conclusion, it appears that there is no causal relationship between IgA-deficiency and hypersensitivity of the chromosomes to

X-rays Disturbance of the immune system in combination with enhanced chromosome breakage after ionizing radiation is apparently typical for AT and AT-like syndromes such as NBS only

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Chapter 9

HIGH INCIDENCE OF SISTER CHROMATID EXCHANGES AT THE 14q1 REGION IN PHA STIMULATED LYMPHOCYTES

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SUMMARY

The distribution of sister chromatid exchanges (SCE's) was studied in PHA-stimulated lymphocytes from seven normal persons and five Bloom's syndrome patients, using a double staining technique. In general the distribution of exchanges between the chromosomes was a function of chromosome length. The localization of SCE's at the chromosome region level, in cultured lymphocytes of normal persons, revealed at the 14q1 region a higher frequency of SCE's than expected . The significance of this finding will be discussed.

INTRODUCTION

The phenomenon of sister chromatid exchanges (SCE) was first demonstrated by Taylor in 1958. After the many years of intensive SCE research it may seem surprising that the biological significance and the mechanism of induction of SCE's still have remained a mystery. The relationship between SCE induction and mutational events, carcinogenesis, chromosome breakage, meiotic recombination and DNA repair mechanisms has been studied, but SCE's do not appear to be clearly related to any of these processes (Wolff, 1978; Hoo and Parslow, 1979; Painter, 1980; Carrano and Thompson, 1982). Also the random or nonrandom distribution of sister chromatid exchanges in individual chromosomes or specific chromosomal regions has been examined extensively (Alhadeff and Cohen, 1976; Morgan and Crossen, 1977; Haglund and Zech, 1979; Hoo and Parslow, 1979; Ockey, 1980;

Vormittag, 1983). The results of these studies are often conflicting. Possibily, the major reason for this is the great technical difficulty to localize SCE's precisely. The simultaneous staining of SCE's and Q-bands that has been used by various authors (Haglund and Zech, 1979; Hoo and Parslow, 1979) or the two step fluorescent staining of Latt (1974), has not allowed the exact positioning of SCE's. The identification of the sites of SCE's has been more succesfull by the use of a double staining technique developed by one of us (J.S.). Using this technique more than 2500 SCE's in PHA stimulated lymphocytes from 7 normal persons could be localized at the region level (The Paris conference in 1971 has defined a chromosome region as any area of chromosome lying between two adjacent landmarks).
Because of the high incidence of sister chromatid exchanges in cultured cells of Bloom's syndrome patients we have attempted to perform the same kind of analysis on their cells. However, the high frequency of SCE's per chromosome in these cells has much hampered an exact localization at the region level. Therefore, the analysis of the SCE's from the cells of Bloom's syndrome patients is limited to their frequency per chromosome.

MATERIALS AND METHODS

Human peripheral lymphocytes from 7 healthy volunteers (two women and five men) and from 5 Bloom's syndrome patients (two girls and three boys) were cultured in RPMI culture medium complemented with 15% fetal bovine serum, antibiotics, PHA and BrdU (20mcg/ml). The cultures were incubated in the dark for 72 hr at

37°C. Two hours prior to harvest colcemid was added. Chromosome preparations were made in the standard manner (Scheres, 1976). In order to get G-banding, slides were stained with Giemsa after a mild trypsine treatment (Seabright, 1971). Good metaphase spreads were photographed under 1000x magnification. The cells were then destained in a 1:3 mixture of acetic acid and methanol. After rinsing in deionized water the chromosome preparations were restained in a 0,1% solution of Basic Fuchsin in a 1:1:1 mixture of 0,1N NaOH:formamide:water(pH 10,2) to obtain a differential staining of the sister chromatids (Scheres, 1977).

All metaphase spreads that already had been photographed (G-banding) were reexamined and again photographed when a clear SCE staining was present. These cells were used for the localization of SCE's .

RESULTS

The number of SCE'S in normal persons was 9.25 per cell (2507 SCE's in 271 cells) with a range of 0-18 while the mean number of SCE's in the Bloom's syndrome patients was 136.37 (12000 in 89 cells).

Both for normal and Bloom's syndrome cells the frequencies of SCE's per chromosome were compared to the relative metaphase chromosome length. The longer chromosomes showed a tendency to have more SCE's than expected and the shorter ones to have less. This is illustrated in Figs. 1 and 2. A linear regression analysis was performed for all paired observations. This was also done for the theoretical line which represents the relative

length of the chromosome according to the Paris chromosome

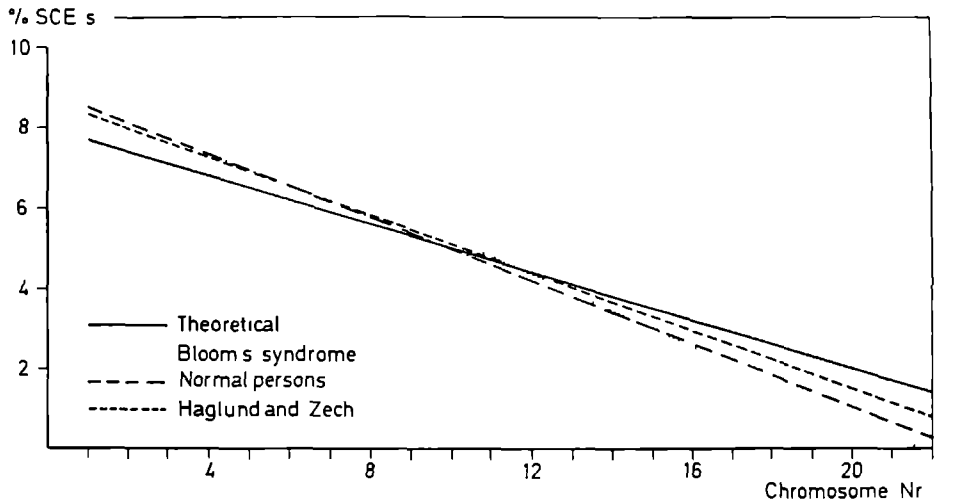

FIGURE 1.
Regression lines of the frequencies of SCE's in each chromosome expressed as percentages of the total. Regression lines are calculated from the data obtained from cells of normal persons, from cells of Bloom's syndrome patients, from data of Haglund and Zech (1979) and from the estimated frequencies (theoretical) of SCE's according to the chromosome length. Only autosomes were taken into account.

conference (1971) and for data collected from the literature (Haglund and Zech, 1979).

From 2507 SCE's, obtained from the chromosomes of PHA stimulated lymphocytes of 7 normal persons, a more detailed analysis at the region level was performed. For practical reasons only the autosomes were taken into account. Results are shown in Fig. 3. For each region the ratio of the observed SCE's to the number of SCE's expected according to their length is given. These data were also plotted in a histogram which indicated a bell-shaped distribution with the region 14q1 outside the X+2SD limit and the short arms of the acrocentric chromosomes outside the X-2SD

limit.

As mentioned before this kind of analysis was not possible in the chromosomes the Bloom's syndrome patients.

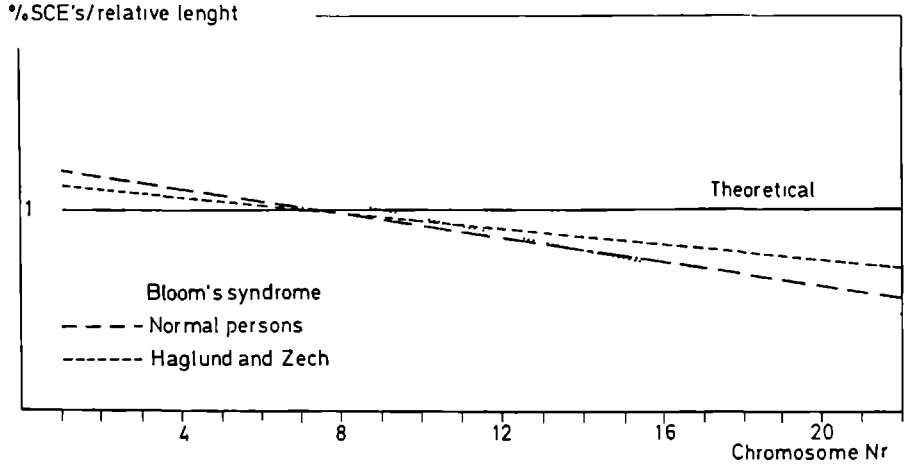

FIGURE 2.
Comparison of the relative frequency of SCE's in cells from normal persons (own observations and from Haglund and Zech 1979) and Bloom's syndrome patients with the frequency assuming that there is a distribution of SCE's according to chromosome length (this frequency is 1 for all chromosomes). It is clearly shown that the longer chromosomes have more SCE's than expected and the shorter ones have fewer.

DISCUSSION

Our data indicate that the frequency of SCE's in human chromosomes appear to be correlated with chromosomal length (Figs. 1 and 2). However, in normal control lymphocytes as well as in Bloom's syndrome lymphocytes the larger chromosomes had more and the smaller chromosomes had less exchanges than expected. Such a deviation from the expected SCE frequency is

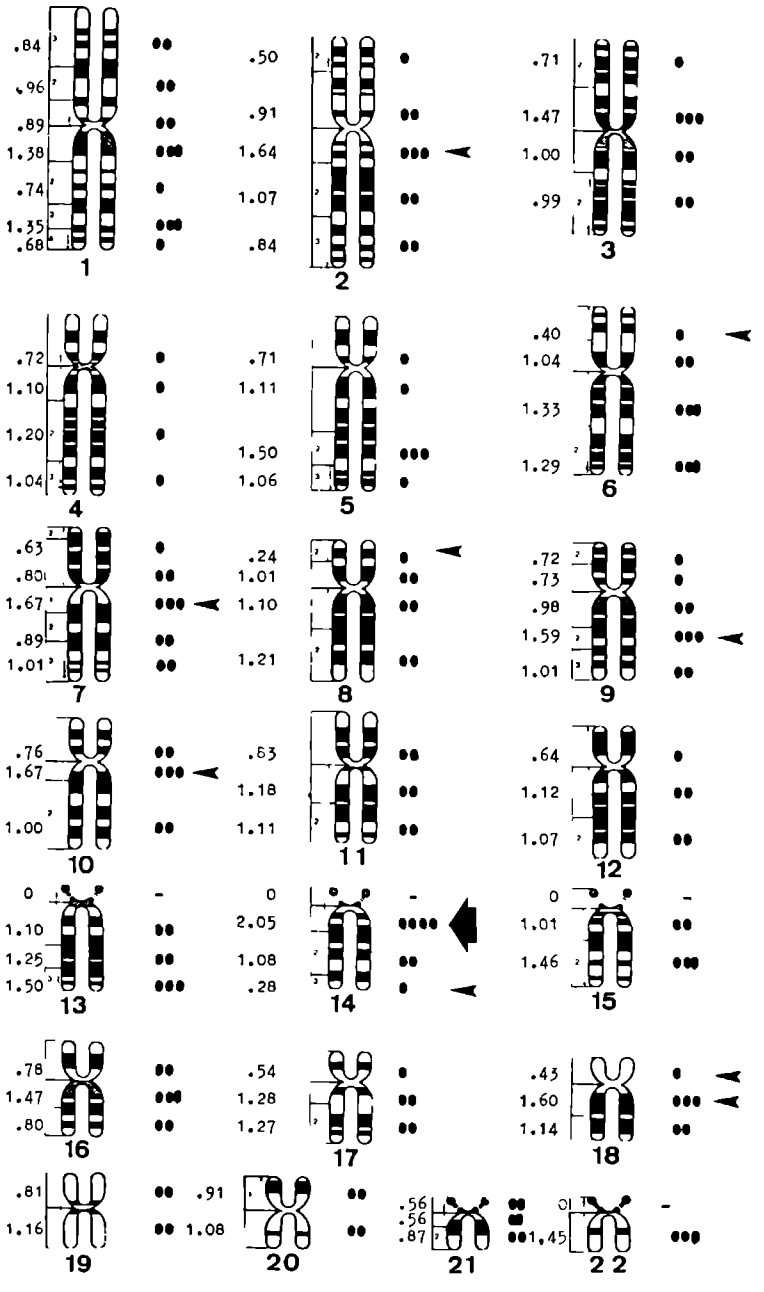

FIGURE 3.
Relative frequencies of SCE's per region. A total of 2507 SCE's in PHA stimulated lymphocytes from 7 normal persons were localized. At one region, 14q1, the number of exchanges was twice the calculated value.

also reported by Aldaheff and Cohen (1976), Morgan and Crossen (1977) and Haglund and Zech (1979). The relatively low freguency of SCE's in the smaller chromosomes may be explained by the low SCE incidence in the centromeric regions which form a large part of these chromosomes (Latt, 1974). Also the technical difficulties in scoring SCE's in smaller chromosomes may contribute to these results. The sites of 2507 SCE's in PHA stimulated lymphocytes of normal persons were determined in relation to the G-banding pattern. Previous attempts to relate the location of SCE's with G-bands have shown that most if not all SCE's appear to be located in the pale staining G-negative bands or at the band junctions (Latt, 1974; Pathak et al, 1975; Haglund and Zech, 1979). In our study this seemed to be true

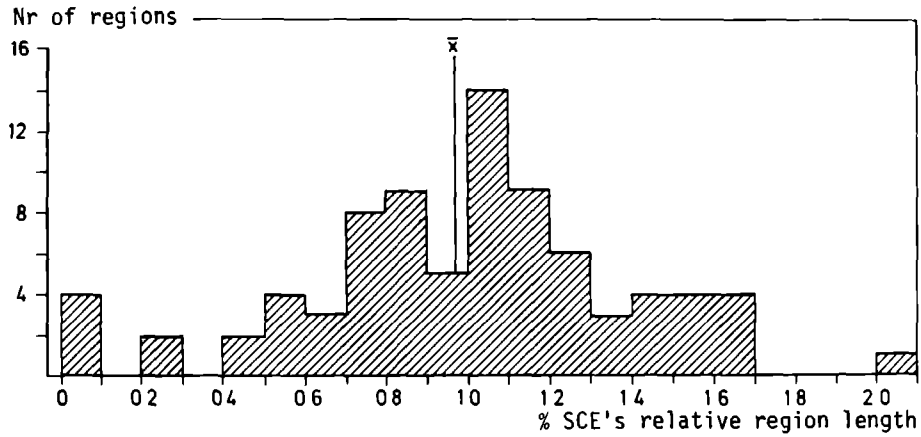

FIGURE 4.
Histogram of relative SCE frequencies showing that five frequencies were outside the X ±2SD (0.97 ±0.82) limit. The four regions with a score of 0 were 13p1,14p1,15p1 and 22p1 and the region with a score of 2 was 14q1.

also, but this implicates that it is sometimes very difficult to assign an exchange to a concrete band. For this reason we decided to localize the SCE's at the chromosome regions instead of the

chromosome bands. At several regions more SCE's were found than expected (Fig. 3) but in only one, 14q1, the number of exchanges was twice the expected value. In addition, the SCE frequency at this site was outside the X+2SD limit (Fig 4). In a normal distribution one would expect that 1 in 20 events is outside the limit of X ±2SD which in our situation means that about 4 regions (the total nr of regions is 79) should fall outside these limits. Indeed five regions appear to be outside these limits but in four, this is not surprising because they represent the short arms of the acrocentric chromosomes 13, 14, 15 and 22 in which it is very difficult to perceive SCE's. Statistically the observed high SCE frequency at 14q1 could be based on chance. However, it is more likely that this observation is an indication of a cellular process occurring at this specific chromosomal region.

It is known that this particular chromosomal site is prone to breakage. In many patients with T-cell malignancies, such as Sezary's syndrome, mycosis fungoides, cutaneous T-cell lymphoma, chronic T-cell lymphocytic leukemia and adult T-cell leukemia a rearrangement of the long arm of chromosome 14 with a break at 14q11-14q13 was reported (Ueshima et al, 1984; Sadamori et al, 1986; Hollis et al, 1987). Based on these findings it has been suggested that the break at 14q11-14q13 probably constitutes a most nonrandom abnormality in T-cell malignancies.

Four specific sites on human chromosomes appear to be preferentially involved in sporadic rearrangements in PHA-stimulated lymphocyte cultures (1 in 1000 metaphases) (Reddy and Thomas, 1985; Dewald et al, 1986; Scheres et al, 1986; Hecht et al, 1987). These breakpoints are in the chromosome bands 7p13, 7q34, 14q11 and 14q32. An analysis from a large number of

translocations between chromosomes 7 and 14 (total no. of 916 breakpoints; results from literature (Scheres et al, 1986; Hecht et al, 1987) has shown that 14q11 is the most common site (47%) to be involved in these sporadic translocations followed by 7p13 (25%), 7q34 (24%) and 14q32 (4%).

The same chromosomal sites are preferentially involved in the very characteristic and highly frequent occurring 7/14 rearrangements in cultured lymphocytes of patients with the chromosome instability disorder ataxia telangiectasia (AT)(Aurias et al, 1980; Weemaes et al, 1984) and of patients with the Nijmegen breakage syndrome (NBS)(Weemaes et al, 1981; Weemaes et al, 1986; Taalman et al, 1987). At the chromosomal bands 7p13, 7q34 and 14q11, T-cell receptor genes are located (Croce et al, 1984; Isobe et al, 1985; Caccia et al, 1984) and these are precisely those regions that are expected to be more accessible to a DNA-recombinase during T-cell development. In NBS and AT it seems that relatively many errors occur in recombinations involving the afore mentioned loci, resulting in a high frequency of 7/14 translocations.

Recently a number of models have been proposed for T-cell antigen receptor and immunoglobulin gene rearrangements. One of these models pertained to unequal sister chromatid exchange (Kronenberg et al, 1987). The high incidence of SCE's at 14q1 in PHA stimulated lymphocytes could result from such exchanges at the highly active T-cell receptor alpha chain genes located at 14q11 (Croce et al, 1985). The events of chromosomal rearrangements at 14q1 and the increased SCE frequency at the same site might thus both be the consequence of cellular recombinational activity.

In view of this it would be of great interest to study the

distribution of SCE's in lymphocytes of NBS and AT patients. It is tempting to speculate that the suspected faulty recombinational activity in NBS and AT lymphocytes which may lead to a high frequency of chromosome translocations (Scheres et al,1986) might also be expressed by a higher SCE incidence at the loci involved.

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Chapter 10

SUMMARY AND CONCLUDING REMARKS

In this thesis studies are presented on a total of five Nijmegen breakage syndrome patients from five unrelated families, two from the Netherlands and three from Czechoslovakia. The clinical, immunological, cytogenetic and cellbiological investigations are described in Chapters 2, 3, 5, 6, 7. Also those from a number of associated topics are included (Chapters 4, 8, 9). The results will be briefly summarized in the following section.

The Nijmegen breakage syndrome (NBS) is a rare inherited condition which is clinically characterized by microcephaly, peculiar face, stunted growth and recurrent infections predominantly of the respiratory tract. Immunological disturbances are presented in all patients varying considerably from patient to patient. Both the humoral and the cellular systems are affected. The immunoglobulin deficiency in NBS patients may be demonstrated by absent or diminished IgA, IgD, IgE and/or IgG. The deficiency of cellular immunity is displayed by a decrease in the percentage of circulating T-cells and/or by an impairment of T-cell proliferative responses (Chapters 2, 5 and 6).

Chromosome instability is another important feature of NBS. Basically the karyotype of the NBS patients is normal but in a proportion of PHA-stimulated lymphocytes structural chromosome aberrations are found. A large number of these abnormalities are reciprocal translocations which involve one, two or even three of the chromosomes 7 and 14. The exchange points in these rearrangements are predominantly located at four 'fragile' sites.

Two in chromosome 7; 7p13 and 7q34 and two in chromosome 14; 14q11.2 and 14q32. Defining the exact breakpoint on 7q has been difficult, formely it was specified as q32 but recent data indicate that the exchange point on 7q is located more distally, at least at 7q34 (Chapters 2 and 6).

In addition the cells from NBS patients appeared to be hypersensitive to X-irradiation. NBS fibroblasts are more readily killed by ionizing radiation than normal cells. Further, X-rays induced more chromosomal damage in NBS cells as compared to normal cells and the DNA-synthesis in NBS cells, was more resistant to irradiation than that in normal cells (Chapters 3 and 6).

In the course of our investigations it became obvious that NBS is closely related to one of the classic chromosome breakage disorders; ataxia telangiectasia. The immunological and chromosomal anomalies in NBS resemble those in AT and the reaction of NBS cells to X-rays is similar to that of AT patients. The DNA synthesis after ionizing radiation in NBS and AT might be a helpful tool in the early diagnosis of both diseases. The differentiation between the two diseases has to be based on clinical symptoms (microcephaly and normal alpha-fetoprotein in NBS versus cerebellar ataxia, telangiectasias and elevated levels of alpha-fetoprotein in AT) and exceptionally by complementation analysis (Chapters 4 and 6). Because of the consistent cellular hypersensitivity to X-rays, present in NBS and AT, it is possible to carry out a genetic analysis by studying the expression of their phenotype in fused somatic cells from different patients. Complementation of the radioresistant DNA-synthesis in heterokaryons (fused cells from

different patients), to levels comparable with that in normal fibroblasts, supports that the fused cells belong to different complementation groups. Such analysis might demonstrate whether NBS is a reflection of the phenotypic variation within AT or if it represents a distinct genetic entity. None of the four NBS patients tested belonged to one of the AT complementation groups. Surprisingly, the NBS patients could be assigned to two separate complementation groups. No correlations between complementation groups, clinical features, chromosomal abnormalities and/or immunological disturbances were noticed. The data obtained from the complementation analysis warrant the conclusion that the patients suffer from a chromosome instability disorder that is distinct from AT. It appears that both NBS and AT belong to a spectrum of radiosensitive human disorders (Chapter 7).

We also studied the hypersensitivity to X-rays in cultured lymphocytes of individuals with selective IgA deficiency. There were no indications that cells from these patients were more susceptible to ionizing radiation. Therefore, a relationship between IgA-deficiency and radiosensitivity, as might be suggested by the frequently occurring coincidence of IgA-deficiency and hypersensitivity to X-rays in NBS and AT patients was not established (Chapter 8).

In the scope of the investigations presented here, attention has been be given to the sites of sister chromatid exchanges (SCE's) in PHA stimulated lymphocytes of normal persons. In one chromosomal region, 14q1, a higher frequency of SCE's than expected was demonstrated. This finding indicates a 'fragility' at this chromosomal site. In view of the frequently occurring chromosome 7 and/or 14 rearrangements in NBS and AT (with one of

the breakpoints at 14q11.2) the same kind of analysis should be performed in cultured lymphocytes of the NBS and AT patients (Chapter 9).

In NBS as well as in AT immunological disturbances, karyotype instability and X-ray hypersensitivity are present. The simultaneous occurrence of these features (albeit with variable expression) in both diseases suggests that they may originate from a defect in a complex fundamental process. Based on our observations and on those of others an attempt will be made to postulate a unifying concept for these radiosensitive disorders. The in vitro radiosensitivity of NBS and AT is universally manifested by a pronounced reduction in the extent of radiation-induced inhibition of the DNA synthesis as compared to that occurring in normal cells. From studies by Painter (1985) it appears that the target for this defect is at the level of individual replicons and affects the inhibition of replicon cluster initiation at radiation damaged regions. It was proposed by Paterson et al (1984) that the deficiency in suppression of DNA replicative synthesis may be the effect of a reduced incidence of a particular class of DNA strand openings that normally serve to inhibit replicon initiation until repair systems have performed their function. In normal cells those chain openings may be introduced by recombinational enzymes which are considered essential for repair of radiation induced DNA lesions (Bridges, 1981; Waldmann et al, 1983, Paterson et al, 1984). These primary signals for radiogenic inhibition of

replicon initiation may be impaired in NBS and AT.

The explanation of immunodeficiency in NBS and AT can also be explained at the molecular genetic level. Immunoglobulin genes as well as T cell receptor genes have to be rearranged before transcription occurs. These processes are quite similar in both gene types (Mak et al, 1985). A deficiency in, for example, an enzyme for splicing could well account for the defects in both B and T-cells which is eventually leading to immunoglobulin deficiency.

Other indications for a possible recombinational defect are the frequently occurring chromosome translocations of the chromosomes 7 and 14 in cultured lymphocytes of our patients. Four chromosomal sites 7p13, 7q34, 14q11 and 14q32 are preferentially involved in these alterations. At 7p13 the T-cell receptor (TcR) gamma chain gene is positioned (Morton et al, 1985) at 7q34 the TcR beta chain is located (Isobe et al, 1985). The gene encoding the TcR alpha chain has been assigned to 14q11 (Jones et al, 1985), whereas band 14q32 contains the immunoglobulin heavy (IgH) chain locus (Wesley Mcbride et al, 1982). The IgH and TcR genes are structurally related to one another and they represent sites where somatic recombination has been shown to take place during normal differentiation and maturation of lymphocytes (Brack et al, 1978, Tonegawa, 1983, Gascoine et al, 1984). Each type of chromosome rearrangement between the aforementioned regions can be explained by errors in molecular recombination.
There are thus indications that the cellular radiosensitivity, the karyotype instability and the immunodeficiency in NBS and AT

are caused by a disturbance in recombination processes. The same enzyme system(s) (cutting and splicing enzymes) might also be crucial in tissue differentiation in utero. A defect of these enzymes could therefore also explain defective organ maturation and production of fetal proteins in AT patients e.g. the elevated level of alpha-fetoprotein describe by Waldmann and McIntire (1972).

Such a presumed mutation resembles the recA mutation in E.coli (Bridges, 1981). The recA protein plays an important role in DNA repair but is also required for cutting and splicing of bacterial DNA (genetic recombination).

If an anomaly in the process of genetic recombination is the primary defect in NBS and AT it should be a very subtle change in DNA processing enzymes because if it is not, the embryo would not survive.

Future research efforts on these syndromes should focus on the relationships between immunodeficiency, chromosomeinstability, radiosensitivity and predisposition to malignancy, because they are probably all tied to such a fundamental process.

Also elucidation of the molecular mechanisms by which the characteristic chromosomal rearrangements may occur, could lead to a better understanding of the aberrant mechanisms of recombination in NBS and AT patients

Further it would be worthwhile to identify (an) abnormal protein(s), involved for instance in the process of recombination, because such an enzyme would allow a gene to be isolated and would greatly facilitate linkage analysis.

Finally localization and isolation of NBS or AT genes would have

great potential for the heterozygote detection and therapy.

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In dit proefschrift worden vijf Nijmegen breuksyndroom patienten beschreven, twee uit Nederland en drie uit Tsjechoslowakije. De klinische, immunologische, cytogenetische en celbiologische studies zijn beschreven in de hoofdstukken 2, 3, 5, 6 en 7. Verder zijn de resultaten van een aantal verwante studies weergegeven in de hoofdstukken 4, 8 en 9

In 1981 werd door ons bij een twaalf jarige jongen met microcephalie, groeiachterstand, mentale retardatie, immuunstoornissen en huidafwijkingen, een nieuw chromosoombreuksyndroom ontdekt. In gekweekte lymphocyten werden allerlei vormen van translocaties tussen de chromosomen 7 en/of 14 gevonden. Hoewel het ziektebeeld overeenkomsten vertoont met de zogenaamde klassieke breuksyndromen, Bloom's syndroom, Fanconi's anemie, ataxia telangiectasia(AT), kan het er op klinische en/of cytogenetische gronden duidelijk van worden onderscheiden. Een ouder, inmiddels overleden, broertje van de patient vertoonde dezelfde symptomen, helaas konden zijn chromosomen niet worden onderzocht. We noemden dit ziektebeeld " Het Nijmegen Breuksyndroom" (NBS)(hoofdstuk 2).
Vergeleken met normale cellen, zijn cellen van de NBS patient bijzonder gevoelig voor rontgenstraling. Ook tegen bepaalde chemische agentia met clastogene eigenschappen (zoals bleomycine) zijn NBS cellen minder bestand. Deze overgevoeligheid uit zich in een sterk verhoogd aantal chromosoomaberraties en in een duidelijk verminderde overleving van, aan bovengenoemde agentia blootgestelde cellen. Opmerkelijk is het effect van

rontgenstralen, of de behandeling met bleomycine, op de DNA-synthese van NBS cellen. De inhibitie van de DNA synthese is aanzienlijk lager dan in normale controle cellen, die een zelfde rontgen of bleomycine behandeling hebben ondergaan (hoofdstukken 3,6 en 7).

Cellen van AT patienten bezitten dezelfde overgevoeligheid voor ioniserende straling en bleomycine. In dit opzicht vertonen NBS en AT dus een bijzondere gelijkenis. Ook cytogenetisch zijn er overeenkomsten. In gekweekte lymphocyten van AT en NBS komen structurele chromosoom aberraties voor: Speciaal de chromosomen 7 en 14 zijn hierbij betrokken (hoofdstuk 4).

Zowel bij NBS als bij AT zijn immunologische stoornissen vastgesteld. IgA- en IgE-deficientie komen het meest frequent voor, evenals stoornissen in de cellulaire immuniteit en in de synthese van specifieke antistoffen (hoofdstukken 2, 4, 5 en 6).

Sinds de beschrijving van de eerste NBS patienten in 1981 hebben wij de gelegenheid gehad om nog vier andere families met klaarblijkelijk hetzelfde syndroom te onderzoeken. Drie uit Tsjechoslowakije en een uit Nederland. In gekweekte lymphocyten van deze patienten blijken dezelfde typische chromosoom 7 en 14 afwijkingen voor te komen, zoals die ook worden aangetroffen bij NBS patient. Ook zijn bij deze patienten stoornissen in de humorale en cellulaire immuniteit vastgesteld, vergelijkbaar met die van de NBS patient. Bovendien bleken de cellen van deze patienten de karakteristieke overgevoeligheid voor rontgenstralen te vertonen. Op grond van deze bevindingen kon worden geconcludeerd, dat deze patienten lijden aan NBS (hoofdstukken 5 en 6).

Omdat in cellen van AT en NBS het fenomeen van de verminderde

remming van de DNA synthese consistent voorkomt, is het mogelijk AT en NBS genetisch te onderzoeken met behulp van complementatie-analyse.Deze genetische analyse werd uitgevoerd in gefuseerde cellen van zes NBS patienten, twee patienten uit Nederland, twee Tsjechische patienten, een patient uit W-Duitsland en een uit de USA. Geen enkele NBS cellijn kon worden ingedeeld bij een van de bekende AT complementatie groepen. Er konden wel twee NBS complementatie groepen worden onderscheiden. Blijkbaar zijn de NBS patienten niet alleen in klinisch opzicht verschillend van ataxia telangiectasia, maar hebben ze ook een eigen genetische entiteit Bovendien blijkt NBS genetisch heterogeen te zijn (hoofdstuk 7).

Uit een studie naar de chromosoominstabiliteit en stralingsgevoeligheid bij patienten met IgA-deficientie blijkt, dat IgA-deficientie alleen geen verhoogde rontgengevoeligheid veroorzaakt. De typerende hypersensitiviteit van AT en NBS cellen voor rontgenstralen is dus, naar alle waarschijnlijkheid, niet gecorreleerd met het voorkomen van IgA-deficientie in deze ziektebeelden (hoofdstuk 8).

Typische chromosoom 7 en 14 afwijkingen worden in gekweekte lymphocyten van AT en NBS patienten aangetroffen (hoofdstukken 2, 4, 5 en 6). Het bijzondere van deze chromosoomtranslocaties is, dat steeds dezelfde breukpunten in de genoemde chromosomen bij deze uitwisselingen zijn betrokken, nl.: 7p13, 7q34, 14q11 en 14q32. Sinds kort is bekend dat in deze breukpunten genen zijn gelegen met immunologische funkties. Opmerkelijk is dat translocaties tussen de chromosomen 7 en 14 niet alleen worden aangetroffen in patienten met AT of NBS. Ook in normale personen komt zo nu en dan een dergelijke translocatie in gestimuleerde

lymphocyten voor (incidentie van 1 op 1000 metafasen).
De 'fragiliteit' van 14q1 blijkt ook uit een studie naar de localisatie van zgn.'spontane zusterchromatide uitwisselingen' in PHA gestimuleerde lymphocyten van normale personen. Er treden tweemaal zoveel uitwisselingen op in 14q1, dan op grond van de lengte van het chromosoom (cq. hoeveelheid DNA) mag worden verwacht (hoofdstuk 9).
In het laatste hoofdstuk wordt een poging gedaan te komen tot een hypothese over het primaire defect van de rontgengevoelige syndromen NBS en AT.

I this way I want to thank my friends and colleagues of the section Cytogenetics, Department of Human Genetics, for their participation in this study. The contributions of the students at the time of the investigations are gratefully acknowledged.

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CURRICULUM VITAE

Rob Taalman werd geboren te Westdorpe op 2 juli 1954. In 1970 haalde hij het diploma MULO-A en B aan te St Clemens MULO te Hulst. Daarna volgde hij een opleiding aan de school voor laboratorium personeel te Breda, waar hij in 1973 het diploma HBO-A analytische chemie behaalde. In 1973 begon hij met de studie biologie aan de Katholieke Universiteit te Nijmegen, waarna hij in september 1977 het kandidaatsexamen aflegde. Het doctoraaldiploma, met als hoofdvak Chemische Cytologie en de bijvakken Farmacologie en Geobotanie, behaalde hij in februari 1981. Van 1 maart 1981 tot 1 maart 1985 (fulltime tot juni 1984, parttime tot maart 1985) was hij als wetenschappelijk medewerker verbonden aan het Antropogenetisch Instituut van de Katholieke Universiteit te Nijmegen. Sedert juni 1984 (eerst parttime, later fulltime) is hij als cytogeneticus werkzaam bij Hazleton Biotechnologies te Veenendaal.

STELLINGEN

Behorend bij het proefschrift:
'The Nijmegen breakage syndrome. Clinical, Immunological, Cytogenetic and Cellbiological studies' van R.D.F.M. Taalman.

I

Het Nijmegen breuksyndroom is nauw verwant aan ataxia telangiectasia, maar het kan er op klinische en genetische gronden duidelijk van worden onderscheiden.
Dit proefschrift

II

In of in de nabijheid van de breukpunten, die betrokken zijn bij de chromosoom 7 en/of 14 translocaties in gekweekte lymphocyten van Nijmegen breuk syndroom- en ataxia telangiectasia patienten, zijn genen gelegen met immunologische funkties. Ook andere translocaties die in de lymphocyten van deze patienten voorkomen, zouden kunnen wijzen naar loci van genen die belangrijk zijn voor het immuunsysteem.

III

Het verhoogde aantal zusterchromatide uitwisselingen in de regio 14q1 is één van de uitingen van instabiliteit op die plaats.
Dit proefschrift

IV

Er is geen causaal verband tussen selectieve IgA deficientie en chromosoominstabiliteit.
Dit proefschrift

V

De chromosomale schade die ontstaat door Rontgenstralen in lymphocyten die van te voren zijn behandeld met een lage Rontgendosering, is aanzienlijk minder dan in niet voorbehandelde cellen.
Shadley and Wolff (1987) Mutagenesis 2:95-97.

VI

Bleomycine of Rontgen gevoelige mutanten van Chinese Hamster ovarium cellijnen zouden een belangrijke rol kunnen spelen in het onderzoek naar de localisatie van de Nijmegen breuksyndroom en ataxia telangiectasia genen

VII

De door bleomycine geinduceerde veranderingen in de collageen synthese zijn afhankelijk van de groeikarakteristieken van de 'target' cellen.
Kirchhofer et al (1986) In Vitro Toxicol 1:55-64.

VIII

Trisomie 21 patienten lopen op de kinderleeftijd meer kans een acute leukemie te ontwikkelen. Het behoeft nadere analyse of de aanwezigheid van een extra Ets-2 oncogen hierbij een rol speelt.

IX

In tegenstelling tot wat de naam suggereert, kan TNF-alfa (tumor necrosis factor alfa) tumor groei stimuleren.

Leibovich et al (1987) Nature 329:630-632.

X

Uit onderzoekingen naar mutaties van het DMD (Duchenne muscular dystrophy) gen blijkt, dat pulsed field elektroforese de bestudering van chromosomale veranderingen bij erfelijke aandoeningen vergemakkelijkt.

den Dunnen et al (1987) Nature 329:640-642.

XI

De uiteindelijke volwassen lengte van patienten met het Turner syndroom kan positief worden beinvloed door behandeling met groeihormoon.

XII

Het geheel eigenhandig willen bouwen van een huis, is zowel de maximale uitdrukking van het streven naar zelfverwerkelijking, als de optimale test van de neiging tot zelfoverschatting.

R.D.F.M. Taalman
16 december 1987

THE NIJMEGEN BREAKAGE SYNDROME

clinical, immunological, cytogenetic and cellbiological studies